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Chemical Attraction of some Marine Fish in their Natural Habitat[1]

A. M. SUTTERLIN

Department of the Environment, Fisheries and Marine Service, Biological Station, St. Andrews, N.B. E0G 2X0

SUTTERLIN, A. M. 1975. Chemical attraction of some marine fish in their natural habitat. J. Fish. Res. Board Can. 32: 729–738.

Using underwater television, photography, and fish traps the attractiveness of a number of natural baits and pure compounds to four species of marine fish was examined directly in their natural habitat. Winter flounders (*Pseudopleuronectes americanus*), mummichog (*Fundulus heteroclitus*), and Atlantic silversides (*Menidia menidia*) were each attracted by specific amino acids. Only flounders and killifish exhibited biting and digging behavior in the vicinity of the odor source. Hagfish (*Myxine glutinosa*) could be trapped using a variety of natural baits but no single amino acid or a variety of combinations of amino acids or amines was effective. The active principle(s) in cod muscle accounting for its attractiveness to hagfish was found to be heat stable, of small molecular weight and extractable with ethanol. Chromatographic separation of the ethanol extract resulted in only one active fraction consisting of several compounds. This fraction, however, accounted for only a small amount of the total activity in the ethanol extract, suggesting a multicomponent system.

SUTTERLIN, A. M. 1975. Chemical attraction of some marine fish in their natural habitat. J. Fish. Res. Board Can. 32: 729–738.

A l'aide de la télévision et de la photographie sous-marines, et de trappes à poisson, nous avons étudié directement l'attrait d'un certain nombre d'appâts naturels et de composés purs à l'endroit de quatre espèces de poisson de mer dans leur habitat naturel. La plie rouge (*Pseudopleuronectes americanus*), le choquemort (*Fundulus heteroclitus*) et la capucette (*Menidia menidia*) sont attirés chacun par des acides aminés spécifiques. Seuls les plies et les choquemorts exhibent un comportement de morsure et de creusage dans le voisinage de la source d'odeur. La myxine du nord (*Myxine glutinosa*) peut être capturée dans les trappes à l'aide d'une variété d'appâts naturels, mais aucun acide aminé ou diverses combinaisons d'acides aminés ou d'amines n'est efficace. Le ou les principes actifs du muscle de morue responsables de l'attraction qu'a ce dernier pour la myxine sont thermostables, à faible poids moléculaire et extractibles à

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[1] A portion of this paper was presented at the Fifth International Symposium on Olfaction and Taste, Melbourne, Australia, October 28–31, 1974.

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l'éthanol. La séparation chromatographique de l'extrait à l'éthanol ne donne qu'une fraction active constituée de plusieurs composants. Cette fraction, cependant, ne rend compte que d'une faible partie de l'activité totale de l'extrait à l'éthanol, ce qui indiquerait un système à composants multiples.

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FEEDING interactions by fish within different trophic levels in the marine environment are mediated by a number of sensory systems. Vision is probably especially important in food finding and selection, but chemoreception by itself or in conjunction with vision is dominant in some marine species (Bateson 1890; Parker 1911; Sheldon 1911; Greene 1925; Pipping 1927; Tester et al. 1954; Bardach et al. 1959; Steven 1959; Aranov 1960; Haynes et al. 1967; Brawn 1969; de Groot 1969). The use of baited hooks in longline, handline, and trap fisheries has been recognized as being effective for a long time (von Brandt 1969). To what extent the success of these natural baits depends on visual and chemical stimuli or both has not always been determined although some work on the catchability of the different natural baits has been carried out in the field (Martin and McCracken 1954).

Some indication of the types of compounds that may be important have been obtained from captive fish and tank studies (Tester et al. 1954; Steven 1959; Bardach et al. 1967; Hodgson et al. 1967; Hashimoto et al. 1968; Konosu et al. 1968; Mathewson and Hodgson 1972). Despite the apparent importance of chemoreception, relatively little work has been done in the field, using artificial prepared chemical baits or pure chemicals in an attempt to attract marine fish (Lindstedt 1971). This study examines the effectiveness of natural bait, bait extracts, and a variety of pure compounds as attractants to four species of marine fishes under natural conditions.

Materials and Methods

Winter flounders (*Pseudopleuronectes americanus*) were observed from the laboratory by means of an underwater television camera situated at the lower edge of the intertidal zone directly in front of the Biological Station in St. Andrews, New Brunswick (Fig. 1A). During the summer months, winter flounders follow the tide into this area, feed in the intertidal zone, and recede with the ebb tide (Tyler 1971).

The field of view of the camera occupied about 1 m^2 of sea bed. Fragments of white clam shells were scattered beneath the camera to improve the contrast of the flounders in relation to the dark bottom; no artificial light was used. A white string was suspended halfway between the ocean bottom and the lens to determine water current direction. During a number of trials a current meter (Marine Advisors Model Q12) was placed near the camera stand and connected to a land-based speed and direction recorder (Model S-13).

Seawater solutions of the compounds to be tested were pumped (50 c^3/min) by a variable speed peristaltic pump through a plastic delivery tubing and released through a spout beneath the television camera. Air bubbles were introduced into the line as a visual indicator of the onset and termination of the chemical stimuli. Testing periods for a single compound consisted of 1 h of pumping of the chemical during the flood tide and 1 h of pumping in the ebb tide. These periods corresponded to water depths of approximately 1.8–3.2 m at the camera stand. Sea water was pumped at a high flow rate following each test to flush residual compounds from the delivery tube. Control tests using sea water and reference tests using glycine, were interspersed among the other compounds tested. All compounds listed were tested at least twice and the order of testing was randomized through the summer season.

For each of the compounds tested the following information was obtained from the variable speed playback of video tape with accompanying voice track: number of flounders observed per hour, direction of approach of flounder entering the camera field, length of time present in the field, and behavior of flounder at the odor source (e.g. biting and digging) and bottom current direction and velocity.

MUMMICHOG AND SILVERSIDES

Field techniques used for mummichog (*Fundulus heteroclitus*) and Atlantic silversides (*Menidia menidia*) are depicted in Fig. 1B. Photographic and pumping equipment were placed behind a blind at the edge of a brackish brook connecting two tidal ponds, both of which are flooded during most high tides. Following high tide, brackish water flowed from pond R_1 to pond R_2. Several large schools of silversides aggregate during ebb tide at the mouth of the brook in pond R_2, probably for the purpose of feeding on plankton leaving pond R_1. It was initially observed that placement of crushed snails and clams in the upper end of the brook resulted in schools of fish entering the brook, locating the food, and feeding on it. Selected chemicals for testing were pumped (1 c^3/min) from behind the blind and released through a hole in a rigid plate of white plastic that rested on the bottom. The number of fish above the plate was determined from photographs taken every minute. Ten-minute control periods, consisting of pumping background water obtained upstream, preceded the 10-min test period using chemicals. The mean number of fish present in 10 frames compared

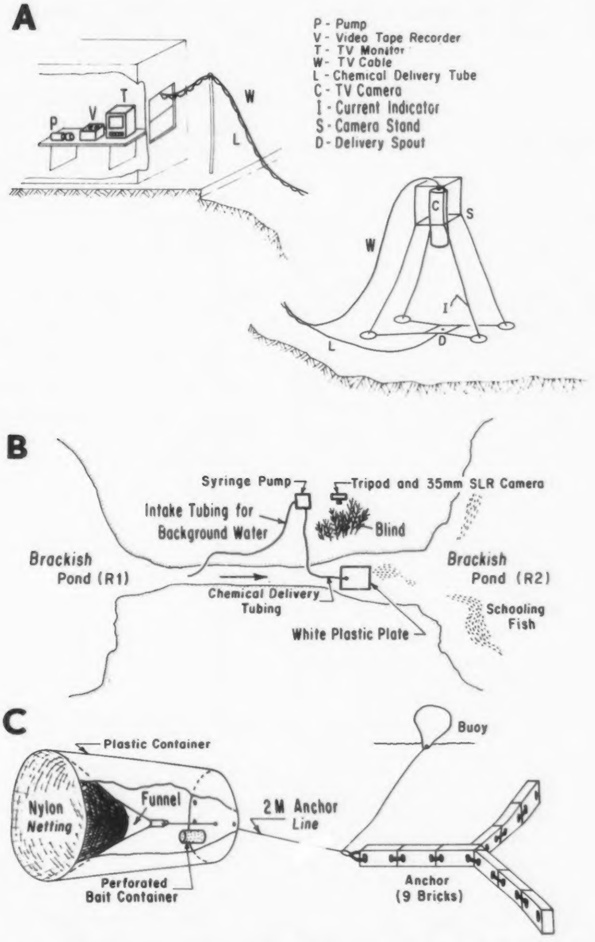
A
P - Pump
V - Video Tape Recorder
T - TV Monitor
W - TV Cable
L - Chemical Delivery Tube
C - TV Camera
I - Current Indicator
S - Camera Stand
D - Delivery Spout
P
V
T
W
L
C
S
W
I
L
D
B
Syringe Pump
Tripod and 35mm SLR Camera
Intake Tubing for Background Water
Blind
Brackish Pond (R1)
Brackish Pond (R2)
Chemical Delivery Tubing
Schooling Fish
White Plastic Plate
C
Plastic Container
Buoy
Nylon Netting
Funnel
2 M Anchor Line
Perforated Bait Container
Anchor (9 Bricks)

FIG. 1. Three experimental designs used in chemical attraction studies. A, Underwater television — winter flounders (*Pseudopleuronectes americanus*); B, photography — mummichog (*Fundulus heteroclitus*) and American silversides (*Menidia menidia*); C, trapping — hagfish (*Myxine glutinosa*).

to those present in the 10 control frames was used as the index of attractiveness of the different compounds tested. Nearly identical methods were used with mummichog except testing was done along the edge of pond (R_1). The frequency of biting at the orifice of the delivery tube was noted for mummichog; no biting by silversides was ever observed.

HAGFISH (*Myxine glutinosa*)

Trapping — A total of 13 hagfish traps (Fig. 1C) were set approximately 100 m apart on mud bottom (depth 45–65 m) in Passamaquoddy Bay, New Brunswick. A rope leader connected the trap to the anchor enabling the funnel end to swing down current. Compounds or bait to be tested were placed in 500-ml plastic bait jars with four holes in the top to permit the release of chemicals within the trap. Traps remained down in most tests for a period of 2½ h. The maximum capacity of each trap was about 425 hagfish. All experiments were conducted in the summer and fall.

Chemical isolation — Freeze-dried codfish extract (FDCE) was prepared by grinding 1200 g of partly frozen codfish fillets into 1 liter of distilled water, stirring the slurry for 15 min, and lyophilizing the filtrate

TABLE 1. Attractiveness of different compounds to winter flounders (*Pseudopleuronectes americanus*) at concentrations of 10^{-3} M[a].

	Trials				
	1	2	3	4	5
Compound	flounders/2 h				
glycine	109	90	81	150	80
L alanine	72	56	40		
L methionine	30	43			
L asparagine	25	33			
L cysteine	21	17			
L glutamic	18	12			
L leucine	10	15			
S.W. controls	0	2	2	3	7
L aspartic	1	4			
L serine	6	2			
L histidine	3	1			
L arginine	0	1			
L threonine	2	0			
taurine	0	3			
L phenylalanine	0	0			
L lysine	3	0			
GABA	1	6			
L tryptophan	0	5			
FDCE (0.39 g/L)	39	43			
trimethylamine	0	4			
dimethylamine	0	1			
betaine	5	7			
glucosamine	5	1			
3 dimethylamino-1-propanol	1	3			
L dimethylamino-2-propanol	0	1			

[a]Concentration refers to that in delivery tubing.

to yield approximately 12.5 g of powder. This powder was placed in bait bottles and tested.

FDCE was serially extracted with hexanes, chloroform, and ethanol, using an extraction thimble in a distillative extractor. The solvents were stripped off using a vacuum rotary evaporator. The residue was redissolved and absorbed on filter paper that was placed in bait bottles and tested after solvent evaporation.

The ethanol extract was separated according to molecular weight by gel filtration (Sephadex G10). Distilled water was used as an eluant in the column (2 × 40 cm). Blue dextran and fluorescein dye were used as markers. Ascending, one directional, paper chromatography (butanol and acetic acid) was used to further separate the ethanol extract. Chromatography paper (Whatman 3 mm) was mounted in a Kymograph drum and the extract (50 mg dry wt/paper) was applied along the bottom of the moving paper using pen and reservoir. After the separation, the edges of the paper were removed and developed. The remaining undeveloped paper was cut into horizontal strips containing the different compounds. The corresponding strips from eight such runs were combined, placed in bait bottles, and tested.

The active zone was eluted with ethanol and analyzed by an amino acid analyzer.

Results

FLOUNDERS

Effectiveness of different compounds — Of the 17 amino acids tested (Table 1) at 10^{-3} M, glycine was the most effective. To a lesser extent, alanine, methionine, asparagine, cysteine, glutamic acid, and leucine were also effective. On a weight basis, FDCE extract was not as effective as several of the amino acids. None of the amines or amino alcohols were attractants for flounders. Although the various compounds were tested, often weeks apart, there is probably good justification for comparing the relative effectiveness of the different compounds because the seawater control runs and the reference compounds (glycine) yielded consistent results throughout the summer. However, during the fall, testing was discontinued because flounders could not be attracted even by raising the concentration of glycine 20 times. This seasonal difference is probably due to a change in a behavioral or physiological state rather than availability of flounders in the intertidal zone (Tyler 1971).

Search behavior — Of the 510 flounders attracted to 10^{-3} M glycine, the majority entered the camera field from the downstream side regardless of whether the tests were run during the flood or ebb tides(Fig. 2). This suggests that the fish had intercepted the chemical plume somewhere downstream during their movement into or out of the intertidal area, and then moved upstream to locate the source. Current velocity (10 cm off bottom) in the vicinity of the camera stand was 7 cm/s in a direction roughly parallel with the shore line.

Most flounders remained in the field of view about 15 s during which time many intermittently circled the spout. Of the 510 flounders attracted to glycine, 56 picked up, often repeatedly, pieces of clam shell and rejected them, and 43 exhibited digging indulations which tended to stir up the sediments. Only 10 flounders actually snapped at the delivery tube spout which was transparent and partly obscured by clam shells.

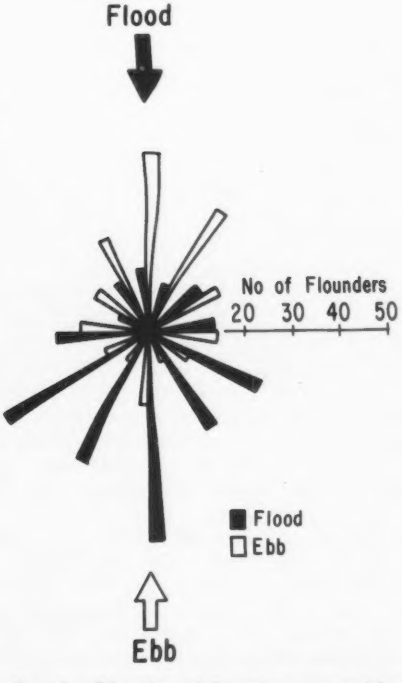

FIG. 2. Direction of flounders approaching an odor source as related to direction of bottom current. (Note: the direction of water current during ebb and flood was not always 180° apposed, as shown in the figure.)

MUMMICHOG AND SILVERSIDES

Amino acids 1–10 in decreasing order were effective attractants for mummichog and several of these compounds initiated biting responses directed at the release orifice (Table 2). Although not noted in Table 2, during the presence of compounds 1–4, there was a pronounced biting and rejection of pieces of debris that had settled on the plastic plate.

Schools of silversides could be consistently induced to enter the brook, using only four of the compounds tested: numbers 2, 5, 6, and 14 (Table 2). No particular feeding behavior (biting, digging, etc.) was exhibited by silversides in the vicinity of the odor source. A great deal of variability in the observations was due to interruption of the experiments by predatory birds (ospreys, kingfishers, gulls, and herons).

HAGFISH

Natural baits — Initial exploratory fishing trials, using several baits, were conducted to locate the concentrations of hagfish in the bay. Muscle tissue from codfish, herring, and trout was effective bait; on a wet-weight basis, shelled clams and shrimp were considerably less effective. To examine the reproducibility among the 15 traps at the selected location, each trap was baited with 150 g of fresh cod fillets and set for approximately 2.5 h. Four control sets, using empty bait bottles, were included. Although there was quite a bit of variability from day to day, and among trap stations, baitless traps failed to catch a single hagfish (Table 3). Trap stations 13, 14, and 15 were in more shallow water and were no longer used in this study as catches were lower, possibly because spider crabs and snails were blocking the funnel.

Pure compounds — Several of the amino acids that were effective attractants for flounders, mummichog, and silversides were placed (dry form) in bait bottles and tested, and not a single fish was caught. More extensive testing of the 24 L forms of the amino acids, shown in the matrix in Table 4A, was carried out. Each amino acid was tested separately, using 0.01 gram molecular weight (GMW) per bait bottle; no hagfish were caught. Various combinations of six amino acids: columns a, b, c, and d, and four amino acids: rows 1, 2, 3, 4, 5, and 6 (Table 4A) were used (0.002 GMW of each compound per bait bottle). No hagfish were caught. A mixture of all 24 L forms (Table 4A) were used at 0.001 GMW and no hagfish were caught. A mixture of 11 amino

TABLE 2. Attractiveness of different compounds to mummichog (*Fundulus heteroclitus*) and Atlantic silversides (*Menidia menidia*) at a concentration of 10^{-4}M*.

	Killifish						Silversides		
	Control**		Test**		Biting***		Tests		
Trial	1	2	1	2	1	2	a	b	c
1. GABA	3	7	68	73	45	68	0	0	0
2. L alanine	1	2	28	63	10	4	208	197	71
3. L histidine	8	0	49	32	22	20	0	0	–
4. glycine	4	10	29	38	38	17	0	0	–
5. β alanine	0	0	19	23	0	1	51	83	33
6. L threonine	2	5	31	15	7	4	25	68	65
7. L leucine	7	9	15	17	0	0	0	10	0
8. L glutamic	4	3	12	20	0	2	0	0	–
9. L isoleucine	0	0	11	15	0	0	0	0	–
10. L valine	8	2	17	9	0	0	–	–	–
11. L norvaline	–	–	–	–	–	–	0	0	–
12. L OH-proline	6	9	7	7	0	0	12	0	0
13. L arginine	3	6	9	0	0	0	0	14	0
14. L methionine	4	5	5	1	0	4	164	93	101
15. L asparagine	6	0	4	6	1	0	0	0	–
16. L lysine	10	3	8	3	0	0	0	0	–
17. L tryptophan	1	7	5	9	0	1	0	0	–
18. L ϕ alanine	8	7	8	5	2	–	0	0	–
19. L aspartic	10	2	8	0	0	0	0	0	–
20. taurine	1	11	10	3	0	4	0	0	–
21. L cysteine	1	9	0	0	3	0	–	–	–

*Concentration refers to that in delivery tubing.
**Mean number of fish per 10 frames.
***Number of fish biting per 10 min.

TABLE 3. Test for reproducibility in catch at 15 trap stations (150 g codfish muscle).

	Catch no. hagfish 2.5 h			
Station	Trial I	Trial II	Trial III	$\bar{M}$
1 control	0	0	0	0
2	83	47	131	87
3	67	0[a]	82	74
4	151	98	79	109
5	203	108	57	122
6 control	0	0	–[b]	0
7	98	75	88	87
8	73	181	–[b]	127
9	3[a]	205	92	147
10	65	113	78	85
11 control	0	0	0	0
12	89	101	128	106
13	43	37	10[c]	40
14	31	21	28[c]	26
15 control	0	0	0	0

[a]Hole in trap netting (data invalid).
[b]Trap destroyed by trawler (data invalid).
[c]Spider crab or snails blocking funnel (data invalid).

acids in the proportions found for free amino acids in carp muscle (Creach 1966) gave zero catch. A variety of other compounds, some of which have been implicated in feeding of aquatic animals, were tested at 0.01 GMW and also proved ineffective (Table 4B). Throughout this period of testing, a number of nonbaited controls were included; catches were all zero. During each set, however, at least one trap contained 1 g of freeze-dried cod extract and catches were consistently over 100 hagfish.

Because of the apparent specificity of something in fish tissue, efforts to characterize the active principle(s) in the freeze-dried extract of cod muscle were carried out. Boiling for 15 min did not alter its activity (Table 5A). No activity was found in either the hexane or chloroform extract; both the ethanol extract and residue were active (Table 5B). Gel filtration of the ethanol extract, after redissolving in distilled water, showed activity only associated with low molecular weight compounds probably below 1000. The only active zone in the separation by paper

TABLE 4. Ineffective compounds tested on hagfish (*Myxine glutinosa*).

A

	a	b	c	d
1	L alanine	L arginine	GABA	5 NH$_2$ valeric
2	L cysteine	L homoserine	L valine	L proline
3	L norvaline	L leucine	L ϕ alanine	OH L proline
4	L histidine	glycine	taurine	L glutamic
5	L methionine	L threonine	L isoleucine	L aspartic
6	L serine	cystine	L tryptophan	L tyrosine

B

trimethylamine oxide	glutathione
trimethylamine	anserine
dimethylamine	carnosine
ethanolamine	cystathionine
glucosamine	1 methyl histidine
betaine	sarcosine
n-butylamine	citruline
n-aliphatic acids (C_3–C_8)	ornithine
AMP′5	asparagine
cytosine	lactic acid
guanine	malic acid
thymine	succinic acid

TABLE 5. Chemical properties of active principle(s) in FDCE tested on hagfish.

A. Heat stability (0.4 g FDCE per trap)

Station no.	Cold	Station no.	Boiled (15 min)
2	42 fish/2 h	1	28 fish/2 h
4	24	3	35
5	54	6	29
7	32	8	33
		10	21

B. Solvent extraction

	5 g FDCE				
	hexanes 0.004 g	chloroform 0.045 g	ethanol 0.609 g	residue 4.280	5 g FDCE
Total activity	0	0	108	214	425[a]
(fish/2 h)	0	0	93	187	368
Specific activity	0	0	177	50	85
$\left(\frac{\text{fish}}{2\text{ h}\times\text{g}}\right)$	0	0	116	44	73

[a] Trap full.

chromatography was zone 3 (Rfs: 0.35–0.50), although its activity was much lower than the activity obtained from the undivided papers when both were placed in bait bottles and tested. Analysis of the ethanol extract of FDCE (Durrum amino acid analyzer) identified 19 common amino acids plus anserine, cystathionine, ethanolamine, and possibly α amino guanido acid. The only active zone separated on paper (Rfs: 0.35–0.50) contained 10 amino acids. Both of the above mixtures were reconstituted in the indicated proportions, dissolved in ethanol, absorbed on filter paper, and tested. No hagfish were caught.

Discussion

It was decided to make observations in natural habitat rather than in the laboratory tanks for the following reasons: Often long periods of adaptation must occur with some fish in captivity before they will exhibit search behavior for food, or even accept food. Once feeding, it is difficult to provide them with the quantity or variety of food normally encountered in the wild. Thus, they may become conditioned to selected food odors, possibly developing associations not usually present in wild fish (Tester et al. 1954; McBride et al. 1962). There is difficulty establishing gradients in tank studies due to confinement in space and limitation in volumes of "clean water." Such conditions might interfere with orientation and search behavior of fish.

On the other hand, each of the field observations using techniques described in this paper suffers in that the exact conditions (i.e. flow rate, tide, species density) cannot be simulated from one experimental trial to the next, necessitating the use of relative rather than absolute units of effectiveness (flounder study). Interspecific interactions (competition) which are probably important in the utilization of a food source often cannot be assessed (hagfish trapping and perhaps in flounder study). There are problems with repetitious observations of the same individual due to limited field of view or large fish aggregations (flounder study, mummichog, and silversides study). Chemical isolation of active components is difficult due to the large batch procedures necessary for assaying in the field (hagfish study). Also such assays are exceptionally time-consuming.

DIET AND FEEDING MECHANISMS

Aquarium observation indicates that flounders and other pleuronectiformes, unlike some flatfish, find food by both chemical and visual means (Pipping 1927; de Groot 1969). Flounders feeding in the intertidal zone in the same area where these studies were conducted, contained in their stomachs algae, amphipods, polychaetes, oligochaetes, and molluscs (Wells et al. 1973).

Parker (1911) suggests that mummichog usually seek food by visual and chemical means, and that the olfactory organ is an important distance-receptor. No information could be found concerning the feeding mechanisms of Atlantic silversides except that their diet is composed of copepods, mysids, shrimp, small squid, and marine worms (Leim and Scott 1966).

The hagfish was chosen for a number of reasons. On morphological grounds the functional significance of Myxine's eye is controversial (Ross 1963; Holmberg 1972). The olfactory capabilities based on laboratory and trapping observations, however, have been commented on by a number of workers (Greene 1925; Gustafson 1935; Ross 1963; Strahan 1963). It is generally concluded that they are not predators of large fish, but will attack dead, injured, or immobilized fish. A variety of bait is effective, however — squid, dead birds (Adams and Strahan 1963). In addition, hagfish stomach contents include a number of invertebrates (Gustafson 1935).

It is probably safe to assume that live, intact organisms (plants and animals) make up the majority of the diet of the species studied here. This is probably less certain for the hagfish, however. The significance behind chemical attraction to nonintact organisms could be explained by considering all three species as opportunistic scavengers. Possibly odors from nonintact organisms as well as many of the pure compounds tested here act not as feeding stimuli but simply as a novel stimuli releasing nonspecific exploratory behavior. A third possible explanation is that these odors are similar to those released by intact organisms that enable fish to locate individual prey or large concentrations of prey. Tank studies have shown that codfish are attracted to odors of some live intact invertebrates and algae (Brawn 1969). Live plankton apparently release an odor that induces exploratory swimming behavior in some plankton-eating fish (Steven 1959). Blinded sharks exhibited a "hunting response" to the odor of a live teleost (Tester 1963).

A number of amino acids were effective attractants for flounders, silversides, and mummichog. Although there is some overlap, different species preferred different compounds. This specificity may have something to do with partitioning food resources.

Amino and imino acids have been implicated in the feeding responses of marine coelenterates (Fulton 1963; Pardy and Lenhoff 1968; Mariscal and Lenhoff 1968), ctenophores (Case and Gwilliam 1963), mollusks (Mangum and Cox 1966; Graham and Mangum 1969; Case and Gwilliam 1963), crustaceans (Case and Gwilliam 1963; Hodgson 1958; and McLeese 1970).

Although some amino acids appeared to be detected by tuna fish, these compounds did not induce the typical feeding response to fish extracts (Tester et al. 1954). Several amino acids and some amines initiated upstream swimming by sharks and some of these compounds could attract captive sharks from some distance (Hodgson et al. 1967; and Mathewson and Hodgson 1972). Steven (1959) tested a variety of compounds on two species of Caribbean shoaling fish held in aquaria; only ammonia, lactic acid, creatinine and glutamic acid initiated exploratory feeding behavior. Bardach et al. (1967) report that cysteine initiated search behavior in the freshwater yellow bullhead (*Ictalurus natalis*).

In contrast to the types of compounds effective for the other three species, our failure to catch hagfish with similar compounds is puzzling. It seems quite certain that no common amino acid acting singularly is effective. Possibly a complex mixture of compounds, including amino acids, along with other unidentified components would be effective.

A more conclusive laboratory study on eels by Hashimoto et al. (1968) and Konosu et al. (1968) showed that, when tested singularly, only 3 out of 18 amino acids were effective attractants. A variety of organic acids, and quaternary ammonium bases were not effective. The attractiveness of extracts of clams to eels could be partially accounted for by the specific proportions of seven amino acids. These papers clearly show the influence of multiple components attributing to the effectiveness of natural baits.

In teleosts, it appears that amino acids, are important components that might contribute to the effectiveness of food odors. It is interesting to note that of a variety of compounds examined electrophysiologically on the olfactory epithelium of a number of freshwater fish, amino acids as a class of compounds stand out as being highly stimulatory (Sutterlin and Sutterlin 1971; Suzuki and Tucker 1971; Hara et al. 1973). Possibly this sensitivity to amino acids in fish has evolved as a food-finding mechanism. High sensitivity by marine fish to these compounds may also be of navigational significance in that it has been shown that the relative proportions of different amino acids distributed vertically and horizontally in oceanic waters differ (Pocklington 1972). If different ocean currents have different odors, such information could possibly be used in initiating, terminating, or assisting fish in their migrations.

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Quantitative and Qualitative Studies of Bacteria Isolated from Sea Water Used in the Laboratory Culture of the American Oyster, *Crassostrea virginica*

R. A. MURCHELANO

National Marine Fisheries Service, Middle Atlantic Coastal Fisheries Center, Pathobiology Investigations, Oxford, Md. 21654, USA

C. BROWN AND J. BISHOP

National Marine Fisheries Service, Middle Atlantic Coastal Fisheries Center, Experimental Biology Investigations, Milford, Conn. 06460, USA

MURCHELANO, R. A., C. BROWN, AND J. BISHOP. 1975. Quantitative and qualitative studies of bacteria isolated from sea water used in the laboratory culture of the American oyster, *Crassostrea virginica*. J. Fish. Res. Board Can. 32: 739–745.

Quantitative and qualitative bacteriological studies were conducted on sea water utilized in laboratory culture of American oyster larvae (*Crassostrea virginica*).

The generic diversity of bacteria isolated from a laboratory seawater storage tank did not change during a 15-day sampling period, although bacterial numbers increased substantially. The mean number of bacteria in ultraviolet light-irradiated sea water was less than that in nonirradiated sea water, although individual samples varied considerably. Over a 10-day sampling period during which oyster larval cultures were sampled repetitively, bacteria were more numerous at 24 h and 48 h than at 0 h.

Pseudomonas was the dominant genus present in stored sea water, irradiated sea water, and sea water from oyster larval cultures.

MURCHELANO, R. A., C. BROWN, AND J. BISHOP. 1975. Quantitative and qualitative studies of bacteria isolated from sea water used in the laboratory culture of the American oyster, *Crassostrea virginica*. J. Fish. Res. Board Can. 32: 739–745.

Les auteurs ont fait des études bactériologiques quantitatives et qualitatives de l'eau de mer utilisée au laboratoire pour la culture de larves d'huître américaine (*Crassostrea virginica*).

La diversité des genres de bactéries isolées d'un réservoir de laboratoire d'eau de mer est demeurée la même pendant une période d'échantillonnage de 15 jours, bien que le nombre de bactéries ait augmenté de façon substantielle. Le nombre moyen de bactéries dans de l'eau de mer irradiée aux rayons ultraviolets est moindre que dans l'eau de mer non irradiée, bien qu'il y ait variations considérables entre les échantillons. Sur une période d'échantillonnage de 10 jours, au cours de laquelle les cultures de larves d'huître furent échantillonnées à plusieurs reprises, les bactéries sont plus nombreuses après 24 et 48 h qu'à 0 h.

Pseudomonas est le genre dominant dans l'eau de mer entreposée, l'eau de mer irradiée et l'eau de mer des cultures de larves d'huître.

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MANY laboratories are able to rear bivalve mollusk larvae under controlled, experimental conditions. Occasionally, larvae which have been feeding and growing steadily suddenly die from no apparent cause. Bacteria, fungi, and protozoa have been implicated as causes of these sudden, catastrophic mortalities; however, they have seldom been isolated from the epizootics attributed to them.

There are few studies specifically relevant to the causes of mortalities in cultures of American oyster (*Crassostrea virginica*) larvae (Davis et al. 1954; Guillard 1959; Tubiash et al. 1965; Tubiash et al. 1970; Brown 1973). Walne (1956, 1958, 1966) and Helm and Smith (1971) have investigated the role of bacteria in cultures of the European oyster (*Ostrea edulis*). With the sole exception of a disease of clam larvae (*Mercenaria mercenaria*) caused by the fungus *Sirolpidium zoophthorum*, documented mortalities of bivalve mollusk larvae have been caused by bacteria belonging to the genera *Pseudomonas* and *Vibrio*.

Since natural sea water is used more frequently

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TABLE 1. Numbers of bacteria (per milliliter) in sea water obtained from six different sources.

	Day			
Seawater source	1	2	3	Mean
Seawater intake	1.5×10^5	2.0×10^5	1.9×10^5	1.8×10^5
Penthouse tank	1.4×10^5	1.7×10^5	2.6×10^5	1.9×10^5
Laboratory tank	5.2×10^4	2.9×10^4	5.5×10^4	4.5×10^4
Cold seawater tap	1.9×10^5	1.5×10^5	2.0×10^5	1.9×10^5
Hot seawater tap	1.7×10^5	3.1×10^4	8.1×10^4	9.4×10^4
UV tap	1.9×10^4	3.2×10^4	1.4×10^4	2.2×10^4

than artificial sea water for culturing oyster larvae, large numbers of generically diverse bacteria are likely to be present. At the Milford Laboratory, oyster larvae are cultured in water from an estuary of Long Island Sound that has a substantial bacterial population, at times higher than that of Long Island Sound proper (Murchelano and Brown 1970). Prior to its use in larval cultures, the water is stored, filtered, and irradiated with ultraviolet light.

This report presents data on the numbers and types of bacteria found in laboratory-treated sea water in oyster larval cultures. Hopefully, such information can be utilized in the selection of prophylactic and therapeutic methods for the control of bacteria in bivalve larval culture.

Methods

Only generalized aspects of the bacteriological methodology will be discussed here; detailed procedures employed and results will be presented where appropriate.

The culture medium (IMI) used for initial isolation of all bacteria consisted of 0.1% yeast extract (Difco)[1], 0.1% trypticase (BBL)[1], and 1.5% agar (Difco) in aged, Millipore[1]-filtered (0.45μm) sea water. All isolations were made by spreading 0.1 ml of sea water on the surface of a suitably dried agar plate. Counts were made from replicate plates. The routine temperature and duration of incubation were 20 C and 7 days, respectively. Maintenance slants were made of IMI medium, and broth medium, when needed, consisted of IMI without agar. The taxonomic scheme employed was essentially that of Shewan et al. (1960). The procedures employed for rearing oyster larvae were basically those of Loosanoff and Davis (1963). All cultures were established with 48-h-old veligers (75 μm diameter) at concentrations of 15 larvae/ml.

Bacterial Flora of Laboratory Sea Water

The laboratory seawater intake is located in an estuary of Long Island Sound that receives considerable tidal flushing. Consequently, the water has most of the biological, chemical, and physical characteristics of Long Island Sound proper. Water at ambient temperature is pumped to a 37,853-liter penthouse settling tank and gravity-fed to the individual laboratories. Heated sea water is obtained using a carbamate heat exchanger operated from the laboratory's boilers. For larval cultures, water is filtered, stored in a 189-liter fiberglass tank, and irradiated prior to use. The maximum flow rate through the irradiation unit is approximately 3 liters/min.

Bacterial numbers in sea water from the laboratory storage tank and in irradiated sea water were significantly lower ($P < 0.05$) than bacterial numbers in sea water from the cold seawater tap (Table 1). There were no significant differences between plate counts made from intake sea water, penthouse tank sea water, and water from the cold seawater tap. Heated sea water did not contain significantly fewer bacteria than cold sea water, and irradiated sea water did not contain significantly fewer bacteria than laboratory tank sea water. Agreement between plate counts of water from a given source and sampled on different days was remarkably good. Although several of the procedures employed lower the bacterial density of sea water, in no instance was it as low as the minimum density noted in Long Island Sound.

Bacterial Flora of Stored Sea Water

Because bacterial density of sea water is lower when freshly drawn, it is customary to fill the 189-liter laboratory storage tank only when necessary to set up or change larval cultures. The tank was filled with filtered sea water and samples were aseptically withdrawn and plated for 14 consecutive days. On each sampling date, repre-

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[1] Reference to trade names does not imply endorsement of commercial products by the National Marine Fisheries Service.

TABLE 2. Bacterial genera (%) isolated from 1) filtered and 2) filtered irradiated sea water in August 1968 and April 1969.

	Sea water			
	Filtered		Filtered, irradiated	
Genus	August '68 (#65)[a]	April '69 (#130)	August '68 (#40)	April '69 (#90)
Pseudomonas	69.2	65.4	75.0	81.1
Achromobacter	12.3	13.1	10.0	3.3
Vibrio	4.6	8.4	0	1.0
Flavobacterium	9.2	6.1	7.5	3.3
Micrococcus	1.5	0	7.5	0
Bacillus	0	0.7	0	0
Yeast	3.0	0	0	0
Unidentified	0	6.1	0	11.1

[a] *Number of bacterial strains isolated.*

sentative bacteria were isolated from the incubated plates and identified.

Although bacterial numbers increased by approximately three orders of magnitude (from 10^4 to 10^7/ml) during the 15-day period, there was no obvious change in generic diversity. The most abundant genus present on the first day maintained its dominance for each of the successive 14 days. Of 165 bacteria cloned during the 15-day sampling, 120 (72.7%) were *Pseudomonas*, 37 (22.4%) *Achromobacter*, 4 (2.4%) *Vibrio*, and 2 (1.2%) were *Bacillus* and *Flavobacterium*, respectively. Pseudomonads were always the numerically dominant bacteria present. We observed no changes in relative abundance of bacterial genera present on the 15 sampling dates.

Bacterial Flora of Ultraviolet Light-treated Sea Water

Each day for 5 days in two experiments and 10 days in another, filtered sea water and irradiated filtered sea water were diluted appropriately and plated. The nonirradiated water was obtained from the same source as the irradiated water by shutting off the UV tubes. Plate counts and isolations were made in August 1968 when the ambient temperature of the estuary water was approximately 22 C and in April 1969 when the ambient water temperature was approximately 4 C. Plate counts only were made in January 1969 when the ambient estuary water temperature was approximately 2 C. At all times the temperature of the water supplied to the irradiation unit varied between 22 and 25 C.

The use of ultraviolet irradiation significantly ($P < 0.05$) reduced the mean number of bacteria present in sea water, although individual samples varied considerably. The numbers of bacteria present in irradiated and nonirradiated filtered sea water in August ranged from 1.0×10^3 to 4.1×10^3/ml (mean — 2.6×10^3/ml) and 2.8×10^4 to 3.6×10^5/ml (mean — 1.7×10^5/ml), respectively. In January there were from 3.4×10^3 to 2.2×10^4 (mean — 1.0×10^4/ml) bacteria/milliliter in irradiated water and from 2.5×10^4 to 9.8×10^4/ml (mean — 5.8×10^4) in nonirradiated water. Bacterial numbers in irradiated sea water sampled in April ranged from 1.9×10^3 to 4.6×10^4/ml (mean — 1.9×10^4/ml) and from 2.2×10^4 to 5.7×10^4/ml (mean — 3.5×10^4/ml) in nonirradiated water.

Pseudomonads were the numerically dominant species present in both irradiated and nonirradiated water in August and April (Table 2). *Pseudomonas* was more numerous in irradiated than nonirradiated sea water; *Achromobacter*, *Flavobacterium*, and *Vibrio*, however, were less numerous after irradiation. The number of isolates obtained from plates of irradiated water was less than from nonirradiated water; pigmented colonies also were found less frequently on plates from irradiated sea water.

Bacterial Flora of Larval Cultures

Four 15-liter plastic buckets were filled with filtered, UV-irradiated sea water. Two buckets were maintained as seawater controls and two were used to culture oyster larvae (15 larvae/ml). All buckets were incubated at 26 C in a constant-temperature water bath. One control and one larval culture bucket were supplemented with 60 mg/liter sulfamethazine every 48 h when the

TABLE 3. Bacterial numbers (per milliliter) in 15-liter cultures containing: 1) sea water, 2) sea water and sulfamethazine[a], 3) sea water, larvae, and food, and 4) sea water, larvae, food, and sulfamethazine[a]. Counts were made daily over a 10-day period during which the cultures were changed 5 times.

	Time					
	0-h		24-h		48-h	
Culture	Mean	Range	Mean	Range	Mean	Range
Sea water	7.2×10^3	3.8×10^3–1.2×10^4	3.2×10^5	1.2×10^4–9.0×10^5	4.6×10^5	3.5×10^5–5.4×10^5
Sea water and sulfamethazine	1.2×10^4	3.1×10^3–3.6×10^4	3.8×10^5	9.0×10^4–6.2×10^5	5.8×10^5	4.5×10^5–7.5×10^5
Sea water, larvae, and food	3.6×10^4	1.5×10^4–6.8×10^4	7.7×10^5	6.0×10^5–9.5×10^5	7.9×10^5	6.8×10^5–9.0×10^5
Sea water, larvae, food, and sulfamethazine	4.8×10^4	1.0×10^4–9.4×10^4	7.4×10^5	5.3×10^5–9.5×10^5	6.9×10^5	4.4×10^5–8.4×10^5

[a] Sulfamethazine $\simeq$ 60 mg/liter.

TABLE 4. Frequency of occurrence (%) of bacterial genera isolated from 15-liter cultures containing: 1) sea water, 2) sea water and sulfamethazine[a], 3) sea water, larvae, and food, and 4) sea water, larvae, food, and sulfamethazine.[a] Isolations were made daily over a 10-day period during which the cultures were changed 5 times.

	Culture			
	Sea water (#238)[b]	Sea water and sulfamethazine (#243)	Sea water, larvae, and food (#244)	Sea water, larvae, food, and sulfamethazine (#231)
Achromobacter	11.3	5.3	4.5	4.3
Flavobacterium	5.0	6.6	10.6	6.9
Pseudomonas	75.6	77.0	77.4	78.7
Vibrio	1.2	1.2	1.6	2.1
Other	6.3	8.6	4.9	7.8

[a] Sulfamethazine $\simeq$ 60 mg/liter.

[b] Number of bacterial strains isolated.

water was changed. Initial plate counts were made on all buckets after additions of sulfamethazine, larvae, and algal food. Subsequent plate counts were at 24 and 48 h and after each water change. Before sampling, the water was thoroughly agitated and the larvae removed by collection on Nitex screens of appropriate size. An initial 1:10 dilution was mixed for 1 min with a vortex mixer before making subsequent dilutions. Counts were continued for a 10-day period during which the cultures were changed 5 times.

In each of the four cultures, counts were significantly lower ($P < 0.05$) in buckets containing 0-h sea water than in the same cultures after 24- and 48-h incubation (Table 3). All cultures containing larvae and algae had significantly higher ($P < 0.05$) counts than cultures of sea water alone. Algal cultures usually are bacterized (Murchelano and Brown 1969) and it is reasonable to assume that oyster larvae themselves are laden with bacteria. There were no significant differences between counts taken at 24 h and again at 48 h. Counts in cultures containing sulfamethazine did not differ significantly from counts in cultures without sulfamethazine.

In all, 956 bacterial isolates were obtained during the 10-day culture period and subsequently identified to genus (Table 4). Pseudomonads were invariably most abundant and accounted for from 75.6 to 78.7% of the bacteria isolated. Least abundant were vibrios, constituting only from 1.2 to 2.1% of the total flora. Flavobacteria and achromobacteria were more abundant than vibrios but considerably less numerous than pseudomonads. No substantial differences existed in the generic percentage composition of the four cultures; sulfamethazine had no apparent effect on the bacterial flora.

The similarity of the bacteria isolated from the four cultures is even more apparent from Table 5. The mean percentage occurrences of specific characteristics for (1) bacteria isolated from seawater cultures and (2) bacteria isolated from cultures containing larvae are remarkably similar. Motile bacteria were substantially more numerous than nonmotile. In the O/F medium of Leifson (1963), oxidative metabolism of glucose was more frequently observed than fermentation. A substantial number of bacteria either produced an alkaline reaction in the medium or effected no determinable change in pH. As is usual with marine bacteria, the isolates were more markedly proteolytic than lipolytic or amylolytic. Overall, the characteristics of the bacteria isolated were in accord with those previously reported by other investigators.

Discussion

Frequent enumeration of the bacteria present in the estuarine sea water supplying the laboratory revealed that bacterial numbers were consistently higher than they were in water from Long Island Sound proper. Bacterial populations ranged from 1.2×10^3/ml to 3.8×10^4/ml (Murchelano and Brown 1970) in a 1-yr biweekly study of Sound water. The characteristics of the estuary on which the laboratory is located produce an organically rich environment in which bacteria flourish. Pumping this water to a settling tank did not reduce its bacterial content.

Bacteria, per se, are not removed by a 15-μm filter but, since invariably some are associated with larger particles, their numbers are reduced. Further, the filter becomes more efficient in removing small particles as it becomes clogged through continuous use. Heating the sea water, especially when the differential between ambient

TABLE 5. Morphological and physiological characteristics (%) of bacteria isolated from 15-liter cultures containing: 1) sea water, 2) sea water and sulfamethazine[a], 3) sea water, larvae, and food, and 4) sea water, larvae, food, and sulfamethazine[a]. Isolations were made daily over a 10-day period during which the cultures were changed 5 times.

	Culture			
Characteristic	Sea water (#238)[b]	Sea water and sulfamethazine (#243)	Sea water, larvae, and food (#244)	Sea water, larvae, food, and sulfamethazine (#231)
Colony size				
Large	15.9	18.5	15.5	19.0
Medium	31.9	33.3	45.0	43.2
Small	44.0	37.0	34.4	32.4
Punctiform	7.9	9.8	4.9	5.2
Colony pigment				
White	52.5	48.1	39.3	39.8
Off-white	20.1	23.0	29.5	27.7
Yellow	16.3	19.3	17.6	15.1
Orange	4.6	2.8	2.4	5.2
Red	0.8	2.0	9.0	6.5
Other	5.4	4.5	2.0	5.6
Gram				
+	0.4	1.2	1.6	0.8
−	97.4	98.0	98.3	94.8
±	2.1	0.8	0	4.3
Motility				
+	83.2	87.6	83.6	89.1
−	16.8	12.3	16.3	10.8
Flagella				
Polar, monotrichous	77.7	84.0	77.0	77.4
Lophtrichous	5.0	3.3	8.2	13.4
Peritrichous	0.4	0	0	0.8
Oxidation/Fermentation				
Oxidative	42.4	47.0	40.1	55.8
Fermentative	1.2	1.2	2.0	3.4
Alkaline	16.8	14.0	17.6	12.5
No reaction	33.2	30.0	35.2	26.4
No growth	5.8	7.8	4.9	1.7
Gelatin	46.2	42.0	37.7	40.7
Starch	23.1	22.0	22.1	27.7
Tween-80	39.0	38.6	30.7	35.9

[a] Sulfamethazine $\simeq$ 60 mg/liter.

[b] Number of bacterial strains isolated.

and the desired laboratory temperature is substantial, reduced bacterial numbers. It is UV-irradiation, however, that caused the most substantial reduction in bacterial density. The ability of UV-irradiation to sterilize sea water is largely dependent on the organic content of the water, flow rate, and the individual efficiency of the irradiation unit. Ultraviolet light irradiation has been used to sterilize sea water with respect to *Escherichia coli* (Kelly 1961; Wood 1961) and poliovirus (Hill et al. 1969). Our UV units do not sterilize sea water and it is probably undesirable to do so. Irradiated sea water appears to contain more pseudomonads and fewer achromobacters, flavobacteria, and vibrios. The reduction in the number of vibrios postirradiation is particularly relevant, however, since it appears that some vibrios cause extensive mortalities in cultures of oyster larvae. Since bacteria were identified to genus only, any changes in species composition resulting from irradiation could not be determined. The utilization of UV-irradiated water in larval cultures will likely continue, not because we know what bacterial (and other) changes result, but because assays with larvae have shown that UV enhances survival and growth.

The numbers and kinds of bacteria encountered in the larval cultures sampled in this study were representative of those encountered in studies conducted repeatedly during the past several years. The number of bacteria present initially was always less than at the time of the first water change (24 h). After 48 h, bacterial populations were only minimally higher than after 24 h. The dominant genus present always was *Pseudomonas*, merely reflecting the dominance of pseudomonads in the filtered, irradiated sea water used in the cultures. Other marine-occurring genera were also present, but in substantially fewer numbers. Although sulfamethazine is used routinely at this laboratory in oyster larval cultures, its ability to reduce the number and types of bacteria present has not been demonstrated.

It is apparent that substantial numbers of metabolically diverse bacteria are present in estuarine sea water. Storage, heating, filtration, and irradiation all effect quantitative and qualitative changes in the bacterial flora present. Since temporal changes also occur (Murchelano and Brown 1970), the bacterial biomass present at any specific time largely is unknown. It is not possible to correlate the success or failure of oyster larval culture with any particular treatment of sea water. Mortalities still occur unpredictably. It is reasonable to assume that under conditions of intensive culture, bacterial numbers will increase substantially and, therefore, should be suppressed. If bacteriological culture and larval bioassay indicate that the bacterial content of the sea water is higher than desirable, some physical treatment to reduce bacterial numbers is desirable. Flow-through or recirculating systems using UV irradiation or ozone should not present major design problems. The number of chemicals which potentially are bacteriostatic, e.g. mercurials, iodines, phenols, etc., is considerable and should be explored for use in hatchery operations. At present, cleanliness is the only expediency that may limit the occurrence of bacterially induced mortalities in cultures of bivalve larvae.

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Utilization of Blue Whiting, *Micromesistius poutassou*, for Human Consumption

BJÖRN DAGBJARTSSON

Icelandic Fisheries Laboratories, Skúlagata 4, Reykjavík, Iceland

DAGBJARTSSON, B. 1975. Utilization of blue whiting, *Micromesistius poutassou*, for human consumption. J. Fish. Res. Board Can. 32: 747–751.

Blue whiting (*Micromesistius poutassou*) is similar to other gadoid species in chemical composition. Eating quality is good and close to that of cod and related species. It is also an excellent raw material for pet food, fish feed, and edible fish powders.

Production of minced block seems to be a more promising processing method than fillet block and applicable machinery is available for this purpose. Minced blue whiting is darker in appearance than most other whitefish products and is stable for only 3 mo in frozen storage. Bleaching adversely affects the eating quality.

Salting and drying of gutted blue whiting as well as air drying of unsalted fish is under investigation.

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La composition chimique du poutassou ressemble à celle des autres gadoïdes. Sa saveur est bonne et se rapproche de celle de la morue et espèces apparentées. Il est de plus une excellente source de matière première de nourriture pour animaux familiers, de farine de poisson et de poudres de poisson comestibles.

La mise en blocs de chair hachée semble une méthode de transformation plus prometteuse que la production de blocs de filets, et une machinerie adaptée à cette fin est disponible. Le poutassou haché a une apparence plus foncée que la plupart des autres produits de poissons blancs et n'est stable que pour une période d'entreposage réfrigéré de 3 mois. Le blanchiment en diminue la saveur.

Le salage et le fumage du poutassou éviscéré, ainsi que le séchage à l'air sont à l'étude.

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THE fish industry of Northern Europe, suffering from dwindling stocks of traditional species, has in the last few years turned its attention to some unconventional sources of raw material. One of those "new" fish species is the blue whiting *Micromesistius poutassou*, a gadoid species closely related to the whiting. Its most common size is about 28–30 cm total length and 120–200 g weight, although individuals over 40 cm and 400–500 g have been caught.

It takes time for the fish trade of the world to accept new fish species, but it should be kept in mind that drastic changes in availability of traditional fish resources may be expected in the next few years, depending on the outcome of the United Nations Conference on the Law of the Sea. Fish technologists and the fish trade may have to adapt to agreements made at international conferences and there will be no time to develop substitute fish products when agreements have been made to ban or reduce catches of traditional fish species.

The purpose of this paper is to evaluate the possibilities of blue whiting in the North Atlantic as a potential food resource in the future.

The Fishery

Biologists estimate at least 8–10 million metric tons of blue whiting in the North Atlantic, allowing annual catches of 1–2 million tons. From March to May the stock concentrates on the spawning grounds northwest of the British Isles and can be easily caught. During summer and early winter the stock is scattered in the Atlantic between Norway and Iceland, and may also be found close to the coast of both countries.

Fishing techniques are still not quite satisfactory, according to recent information (S. Sveinbjörnsson, Marine Research Institute, Reykjavík personal communication). Because blue whiting are usually trawled from at least 250 m, their swimbladders inflate greatly as the trawl is brought to the surface, sometimes causing the trawl to

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rupture. However, fishermen have now found ways to cope with the problem (Jakupsstovu 1974).

Some European nations already maintain a limited blue whiting fishery. Factory trawlers of the Soviet Union are believed to be catching increasing quantities of blue whiting in the northeast Atlantic. According to official statistics total Russian catches of blue whiting were 63,700 tons in 1971. No official estimates are available for later years.

Spanish trawlers catch at least 10–20,000 tons of blue whiting yearly, mainly for fishmeal production (Anon. 1974a).

In Norway and Denmark significant amounts of blue whiting are landed with the so called "Industry fish mixture" for fishmeal plants. According to the Norwegian Marine Research Institute the content of blue whiting in this mixture may amount to more than 100,000 tons yearly.

To test processing methods and marketing on a significant amount of this raw material, the Norwegian government promoted experimental blue whiting fisheries in spring 1974 on the spawning grounds by offering good prices (1 Nkr/kg or about 20¢/kg) for iced blue whiting landed in Norway. In spite of this official campaign only four or five boat owners showed interest and only a few hundred tons of iced blue whiting were landed in addition to some 3000 tons for fishmeal production.

In Great Britain efforts of industry and government to promote blue whiting fisheries this spring were not successful, in spite of very favorable initial reaction.

In Iceland blue whiting landings have been insignificant until now, only a few thousand tons yearly. Fishermen have not found it sufficiently rewarding to catch this species for fishmeal production and the freezing industry has not yet been ready to accept this raw material. In summer 1974 a few hundred tons were accidently caught in conventional bottom trawls, off the south coast of Iceland where the species seemed to be abundant.

Little or no information is available about blue whiting fisheries of other European nations. It is unlikely that they are of any significance yet.

Chemical Composition

Table 1 shows some of our analytical data on blue whiting samples caught in the waters around Iceland. Fat was determined by 4 h of extraction of sodium sulphate dried sample with ethyl ether in soxhlet apparatus, and water content by 4 h of drying at 105 C of sample mixed with analytical sand. Protein determinations were carried out by the Kjeldahl method and amino acids of hydrolyzed raw flesh on a Beckman Amino Acid Analyzer, Model 120 B. Vitamins were determined according to Methods of Vitamin Assay (1966). Analyses reported in Table 1 were carried out on whole, ungutted fish intended for fishmeal production. The differences in fat content (Table 1) may be partly due to the liver which varies in size and oil content and acts as an energy reserve for the fish, or they may be attributable to prey organisms in the fishes' stomachs. The flesh is rather lean, although it contains somewhat more fat than cod and haddock. Table 2 shows that the protein content is almost identical to that of cod fillets, and other nutrients are in extremely good agreement with those of cod and other whitefish species.

Storage Stability Prior to Processing

Since blue whiting is usually caught some distance from the nearest harbor it was necessary to test the storage stability of ungutted fish on ice. Fish that had just been feeding on zooplankton could be stored less than 2 days, but with empty or nearly empty stomachs the blue whiting remained unspoiled for at least 1 wk on ice (Table 3).

Results of Norwegian storage studies using refrigerated sea water have not been very encouraging (Karsti 1974). Blue whiting stored for 4 days in RSW was of low quality with trimethylamine content of 6 TMA-N mg/100 g. The same author found iced blue whiting still good after 7 days of storage.

Blue Whiting Products

Most of the blue whiting caught in the North Atlantic has until now been used for fishmeal, although blue whiting products for human consumption have been marketed in the Soviet Union.

The eating quality of blue whiting is excellent. In several taste tests at the Icelandic Fisheries Laboratories, steamed, baked, or fried fillets of blue whiting and fishcakes from minced blue whiting always received high scores, although lower than corresponding products from cod and haddock.

Similar results were obtained by the English (Anonymous 1974b) and Norwegians (Skilbrei 1974). Scandinavian processors greatly appreciate the binding properties of blue whiting flesh for the production of traditional Scandinavian fish balls. Some British "fish and chips" dealers claim very good results when they use blue whiting fillets in their products. The size of the fillets 45–60 g (1½–2 ounces) is apparently well suited for this purpose.

TABLE 1. Some analytical data on whole, ungutted blue whiting (*Micromesistius poutassou*). Sample size: 20–100 fishes.

Day of catch	Mean TL (*cm*)	Mean wt (g)	Water (%)	Fat (ethyl ether) (%)
Jan. 4	31.9	192	76.5	5.4
May 24	29.4	141	78.0	3.9
” 31	29.5	124	79.3	2.2
June 1	30.0	142	79.0	3.1
” 10	31.0	154	77.7	3.5
” 13	29.9	144	78.4	3.6
” 20	27.6	123	79.0	3.8
July 6	27.9	126	76.2	4.7
” 18	25.3	113	77.9	5.1
Aug. 30	—	—	73.5	9.7
Sept. 9	27.0	124	73.2	9.1
Oct. 4	27.0	143	73.3	8.8
” 15	31.3	237	76.6	12.1
Nov. 1	29.2	135	74.8	5.7
Dec. 3	30.3	187	76.2	5.2
Average	29.0	142	76.6	5.3

TABLE 2. Chemical composition of blue whiting and cod fillets.

Constituent	Blue whiting		Cod	
Water (moisture)	80.5	%	81.1	%
Protein (N × 6.25)	18.1		18.1	
Fat (ethyl ether)	0.8		0.2	
Ash (minerals)	1.2		1.0	
Salt (NaCl)	0.1		0.2	
Calcium	24	mg/100 g	20	mg/100 g
Sodium	51		70	
Iron	1.0		0.9	
Thiamine	0.08		0.06	
Riboflavin	0.09		0.09	
Niacin	1.84		2.1	
Lysine	9.33	% of protein	8.52	% of protein
Histidine	2.67		2.21	
Arginine	5.64		5.52	
Aspartic acid	7.16		9.89	
Threonine	3.21		4.05	
Serine	3.24		4.05	
Glutamic acid	13.12		15.37	
Proline	2.52		3.11	
Glycine	3.50		4.30	
Alanine	5.05		5.54	
Valine	4.36		4.19	
Isoleucine	4.22		4.33	
Leucine	7.86		7.46	
Tyrosine	3.24		3.24	
Phenylalanine	3.82		3.67	
Cystic acid	1.26		1.11	
Methionine	3.31		3.05	

TABLE 3. Storage life of whole blue whiting on ice.

Days of storage	Organoleptic grading of raw material	Cooked flavor[a]	TMA-N mg/100 g
0	Fresh	5.0	0.1
1	Fresh		0.1
3	Fresh, slight "fishy" odor	4.0	0.4
4	Slightly rancid intestines		1.2
5	Rancid liver, soft bellies	3.8	1.4
6	Rancid liver, soft bellies		2.1
7	Spoiled intestines, fillets still good	3.1	2.8
8	Bellies ruptured, off odors in fillets	2.3	5.5
11	Spoiled, putrid		13.6

[a] 5-point scale; 5 = excellent, 2 or below = unacceptable.

The most promising application of the blue whiting seems to be the minced block or fillet block, however. The small size of blue whiting has been an obstacle with regard to processing costs and discouraged processors.

Other possible uses of blue whiting as food are under investigation at the Icelandic Fisheries Laboratories. Headed and gutted fish has been brine salted and dried as "klipfisk." This product tastes very similar to salted cod but the bones may become a serious defect when marketed.

Air drying of blue whiting in conventional stockfish fashion has also been tried with promising results. This product, as well as the salted one, will soon be introduced to market experts.

In Norway, experiments have been conducted to produce pet food and fish feed from whole blue whiting. Both products are said to be of very good quality (Skilbrei 1974).

Blue whiting is also a very good raw material for production of high protein fish powder or fish protein concentrate. Nobody doubts the high nutritional qualities of both these products, but there is still no potential market for such edible fish powders.

Machine Dressing and Processing of Minced Block

Obviously conventional machines have to be modified for heading and gutting, and if desired, filleting of this fish. At least two European makers of fishworking machinery, ARENCO and BAADER have made remarkable progress in modifying their equipment to fit these purposes (Christians and Ritter 1974).

Machine filleting was studied extensively by Norwegian fish technologists in 1973 (Karsti 1974). Filleting and skinning machines that were tested apparently needed some improvement. Capacities were comparatively low and investment costs rather high compared to yield and product value. The skinning operation was especially difficult.

Heading and gutting machines, originally designed for herring belly cutting and filleting, have given better results. The head is cut off and the body cavity completely cleaned with special grinding stones, brushes, and water nozzles that remove the black belly membrane and kidney. After this cleaning procedure, the fish may be put through a deboning machine.

According to Norwegian results based on headed and gutted fish (Skilbrei 1974), the yields vary from 38 to 55% minced flesh depending on size of the fish, type of machine, etc. Christians and Ritter (1974) reported yields of 43% minced flesh based on whole fish. They used a BAADER 694 deboning machine with 5- or 8-mm drum holes. Experiments at the Icelandic Fisheries Laboratories using a YANAGIA bone separator showed that 3-mm holes had to be used to avoid excessive bone contamination. In these experiments yields of 39% minced flesh were obtained from whole frozen blue whiting but 45% when the raw material was fresh. The difference is probably due to drip losses. In both instances heading and gutting were done manually.

Inevitably some blood is squeezed out of the backbone and fins and this is reflected in the color of the minced product. The dark spinal cord also gives it a rather unpleasant appearance. Furthermore the flesh is darker than that of cod and haddock, similar to the flesh color of saith. This is considered a drawback even though the color difference is hardly noticeable after cooking. Some experiments have been carried out to bleach or brighten the color of minced blue whiting.

Washing with water does not improve the appearance sufficiently (Christians and Ritter 1974). The reddish discoloration faded but the intrinsic dark flesh color remained unchanged.

Bleaching and Frozen Storage of Minced Blue Whiting

Experiments at the Icelandic Fisheries Laboratories showed that a good bleaching effect was

Table 4. Quality changes in minced blue whiting during frozen storage at −25 C.

Sample	P.O.V.[a] changes with storage time		
	0 days	21 days	90 days
1[b]	1.0	14.0	73.3 rejected
2[c]	40.5	92.7 rejected	
3[d]	44.2 rejected	—	
4[e]	31.9	27.3	0 rejected

[a] P.O.V. = peroxide value, milliequivalent per kilogram extracted oil.

[b] Sample 1 = untreated.

[c] Sample 2 = bleached in 3.5% peroxide in 0.1% ammonia + 0.1% acetic acid.

[d] Sample 3 = bleached in 3.5% peroxide in 0.1% ammonia + 0.1% ascorbic acid.

[e] Sample 4 = bleached in 3.5% peroxide in 0.1% acetic acid.

obtained by using 3–5% hydrogen peroxide. After alkaline bleaching, the product was washed with 0.1% acetic acid or 0.1% ascorbic acid to remove free ammonia and finally washed with ice water. Bleaching times of ½–2 min were used. After bleaching, the product was light in color, but the characteristic flavor was lost and the texture had toughened. In fish sticks this raw material was only marginally acceptable. After being mixed with spices and other ingredients, bleached blue whiting was still a good raw material for fish cakes.

In a storage stability test a rancid off-flavor was detected in samples neutralized with ascorbic acid immediately after treatment (Table 4). Samples 2 and 4 were rejected because of rancidity and spongy and gritty texture after 21 and 90 days, respectively. In sample 4 no peroxides were detectable by the Wheeler method (American Oil Chemists Society 1950) after 90 days at −25 C although the rancid flavor was objectionable.

Another rather unpleasant finding was that distinct rancidity had developed in the untreated control samples (1) and 3 mo of frozen storage at −25 C. A storage life of less than 3 mo must be considered a serious handicap for a frozen fish product. It is possible that the blood residue catalyzes the rancidity process, and blood contamination should therefore be avoided or the blood removed.

Bleaching with hydrogen peroxide apparently does not solve the rancidity problem and the undesirable properties acquired by bleaching do not seem to justify such treatment for the sole purpose of brightening the color. Further experiment with washing and frozen storage of minced blue whiting are being carried out.

Conclusions

The blue whiting is at least one of the most important, if not the most important, of the unexploited fish resources of the northeast Atlantic. Minced blue whiting block may never be quite comparable in quality to minced cod or haddock. The color will be darker and the storage stability may be considerably less. Nevertheless, it is felt that minced blue whiting will be an important item in the frozen fish trade in the near future. Both the catching and processing techniques seem to be at the breakthrough point.

Marketing of the products remains uncertain. The success of the Alaska pollack block on the American market shows, however, that there is room for unconventional fish items, even though they may be considered of lower quality than the traditional products. The shortage of cod will necessitate a substitute and the blue whiting may be a promising candidate.

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Effects of Acute Exposure to Bleached Kraft Pulpmill Effluent on Carbohydrate Metabolism of Juvenile Coho Salmon (*Oncorhynchus kisutch*) During Rest and Exercise

D. J. McLeay and D. A. Brown

Division of Applied Biology, B.C. Research, Vanvouver, B.C. V6S 2L2

McLeay, D. J., and D. A. Brown. 1975. Effects of acute exposure to bleached kraft pulpmill effluent on carbohydrate metabolism of juvenile coho salmon (*Oncorhynchus kisutch*) during rest and exercise. J. Fish. Res. Board Can. 32: 753–760.

In the static study (no exercise), liver glycogen stores were unchanged during 12-h exposure to 0.8 of the 96-h LC50; longer exposures caused a progressive decrease to levels one fifth those of controls at 72 h. Plasma glucose levels in fish held in 0.8 LC50 effluent for 3–96 h were elevated; at 96 h, glucose had increased threefold. Mean values for plasma lactate were elevated significantly at 3, 6, 24, 72, and 96 h.

In the exercise (swimming one body length per second)–rest study, muscle glycogen levels decreased 53–78% during exercise in water or effluent (0.7 LC50) for 4–12 h, and did not recover during 12-h rest in water. Muscle glycogen for fish exercised for 12 h in effluent and then rested for 4 or 12 h in effluent was lower compared to values for fish exercised in effluent and then rested in water. There was no difference in liver glycogen levels of fish exercised in effluent or water for 4–12 h. Values of liver glycogen for fish exercised in effluent for 12 h and then rested for 4, 8, or 12 h in effluent decreased 60–70% compared to fish exercised in water for 12 h and then rested in water and by 55–65% from fish exercised in effluent for 12 h and rested in water for 4–12 h. Plasma glucose levels were elevated one- to fourfold during exercise in water or effluent. Fish resting in water for 4, 8, or 12 h following exercise in water had relatively stable glucose levels; whereas for fish exercised and then rested in effluent the glucose levels increased twofold during resting. Plasma lactate levels were elevated five- to sixfold during exercise in water or effluent for 4–12 h, declining to values 1–2 times those of stock fish within 4-h rest. Plasma lactate levels for fish exercised in effluent and then rested in effluent or water were continually higher than those for fish exercised and rested in water.

It was concluded that measurement of carbohydrate metabolites, particularly blood sugar levels, in unexercised fish could prove useful as a rapid method for measuring toxicity of pulpmill effluents and other pollutants.

McLeay, D. J., and D. A. Brown. 1975. Effects of acute exposure to bleached kraft pulpmill effluent on carbohydrate metabolism of juvenile coho salmon (*Oncorhynchus kisutch*) during rest and exercise. J. Fish. Res. Board Can. 32: 753–760.

Dans l'étude statique (sans exercice), les approvisionnements de glycogène hépatique demeurent inchangés durant une exposition de 12 h à 0.8 de la CL50 après 96 h; des expositions plus longues entraînent une diminution progressive vers des niveaux d'un cinquième de ceux des témoins à 72 h. Les niveaux de glucose plasmique chez des poissons maintenus dans un effluent de 0.8 de la CL50 durant 3–96 h augmentent; à 96 h, le glucose triple. Les valeurs moyennes du lactate plasmique sont significativement plus élevées à 3, 6, 24, 72 et 96 h.

Dans l'étude exercice (nage d'une longueur de corps par seconde)–repos, les niveaux de glycogène sanguin diminuent de 53–78% durant l'exercice dans l'eau ou l'effluent (0.7 CL50) d'une durée de 4–12 h et ne se rétablissent pas durant un repos de 12 h dans l'eau. Le glycogène du muscle, chez des poissons qui ont pris un exercice de 12 h dans l'effluent suivi d'un repos de 4 ou 12 h dans l'effluent a une valeur inférieure à celle des poissons dont l'exercice eut lieu dans l'effluent et qui se sont ensuite reposés dans l'eau. Il n'y a pas de différence dans les niveaux de glycogène hépatique de poissons qui prennent leur exercice dans l'effluent ou l'eau sur une période de 4–12 h. Le glycogène hépatique des poissons qui prennent leur exercice dans l'effluent durant 12 h et qui se reposent ensuite durant 4, 8 et 12 h dans l'effluent diminue de 60–70%, comparativement aux poissons qui prennent leur exercice dans l'eau durant 12 h et se reposent ensuite dans l'eau durant 4–12 h. Les niveaux de glucose plasmique s'élèvent de 1 à 4 fois au cours de l'exercice dans l'eau ou l'effluent. Les poissons se reposant dans l'eau durant 4, 8 ou 12 h après

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un exercice dans l'eau ont des niveaux de glucose relativement stables; par ailleurs, pour les poissons qui prennent leur exercice et se reposent dans l'effluent les niveaux de glucose doublent durant la période de repos. Les niveaux de lactate plasmique s'élèvent de 5 à 6 fois durant un exercice de 4–12 h dans l'eau ou l'effluent, pour s'abaisser à des niveaux de 1 à 2 fois ceux des poissons de réserve en dedans de 4 h de repos. Les niveaux de lactate plasmique, chez des poissons qui prennent leur exercice dans l'effluent et se reposent ensuite dans l'effluent ou dans l'eau sont toujours plus élevés que ceux des poissons qui prennent leur exercice et leur repos dans l'eau.

Nous en concluons que les mesures de métabolites d'hydrates de carbone, surtout les niveaux de sucre dans le sang, chez des poissons non soumis à un exercice, pourraient s'avérer utiles pour une détermination rapide de la toxicité d'effluents de papeterie et autres polluants.

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MANY investigators have observed changes in the carbohydrate metabolism of fish exposed to a variety of stressors including exercise (Black et al. 1960, 1961, 1962), physical disturbance (Nakano and Tomlinson 1967), starvation (Stimpson 1965; Black et al. 1966), and environmental hypoxia (Heath and Pritchard 1965; Narasimhan and Sundararaj 1971). However, little is known concerning the effects of toxicants on the carbohydrate metabolism of fish. Since both catecholamines and adrenocorticosteroids which are secreted in increased amounts due to stressful stimuli (Nakano and Tomlinson 1967; Fagerlund 1967) elicit marked changes in the carbohydrate energy reserves of fish (Stimpson 1965; Nakano and Tomlinson 1967; Swallow and Fleming 1970; Laarson 1973), it was thought that exposure to sublethal concentrations of toxicants, if stressful, should evoke similar changes.

Although a number of methods are now available to measure sublethal effects of pollutants (Sprague 1971), most are long-term and thus are not suitable for routine monitoring programs. In our laboratory, we are attempting to develop a short-term sublethal bioassay for kraft pulpmill effluents which may ultimately replace the acute lethal bioassay (Howard and Walden 1974). This paper describes two studies carried out to determine the effects of acute exposure to a high sublethal concentration of neutralized, bleached kraft pulpmill effluent on a number of carbohydrate metabolites in juvenile coho salmon (*Oncorhynchus kisutch*), in order to determine time–response and magnitude of response relationships. Experimental design was such that test procedures were simple enough to be applied to routine monitoring of sublethal toxicity of effluents.

In a static study (fish not exercised), the effect of holding fish in pulpmill effluent for 1–96 h on their liver glycogen, plasma glucose, and plasma lactate levels was measured. An exercise–rest study examined the effects of periods of exercise and rest in pulpmill effluent and water on muscle and liver glycogen reserves and plasma glucose and lactate levels.

Static Study

MATERIALS AND METHODS

Test fish — Juvenile coho salmon, *O. kisutch*, obtained from the Washington State Samish Hatchery as swimup fry in the spring of 1971, were held at low population density in dechlorinated tap water (11 ± 1 C, pH 6.6 ± 0.1, conductance 10–30 micromho/cm, EDTA hardness 2–10 mg $CaCO_3$/liter, alkalinity 3–9 mg $CaCO_3$/liter) for several months. The fish, maintained under natural photoperiod conditions, were fed a commercial pellet fish food (O.M.P.) 2–5 times daily. A random sample of 12 fish measured 9.1 ± 0.2 cm and weighed 9.0 ± 0.4 g at the time of this study.

Test effluent — A single supply of neutralized, filtered whole mill bleached kraft effluent (BKME) was used in this study. Effluent characteristics included 96-h LC50 55%, conductance 980 micromho/cm, 5-day biochemical oxygen demand (BOD_5) 150 mg/liter, total organic carbon (TOC) 240 mg/liter, color 2880 APHA units, and sodium 175 mg/liter.

Experiments — Twelve fish were added to each of 20 polyethylene tanks. Each tank measured 45 × 35 × 40 cm, was fitted with a lid and nylon mesh basket and contained 40 liters of dechlorinated tap water. After 48 h, effluent was added to the odd-numbered tanks and water added to the even-numbered tanks, in equal amounts. The volume of effluent added gave a concentration of 0.8 of the 96-h LC50 value as determined by Standard Methods (American Public Health Association 1971) with fish loadings of 2 g/liter and 24-h replacement of test solutions.

All fish in each tank were sacrificed at intervals of 1, 3, 6, 9, 12, 24, 48, 72, and 96 h after the addition of effluent or water. An additional fish sample was taken directly from the stock tank.

The solutions in each tank were renewed by siphoning 90–95% of the volume and refilling through a hole in the lid every 24 h. Water temperature was held at 11 ± 1 C; solutions were aerated minimally to maintain the dissolved oxygen content at saturation. The fish were not fed following transfer to tanks, and considerable effort was made to keep disturbance to fish at a minimum.

Sampling — After individual fish had been netted, the caudal peduncle was dried, cleaned with 95% alcohol, and severed. Blood collected into heparinized microhematocrit tubes was centrifuged at 12,500 rpm for 5 min and stored at −20 C until analyzed. The liver was removed and digested in 30% KOH. Less than 15 min were required to sample all the fish from each tank.

Analyses — Liver glycogen was precipitated by the method of Good et al. (1933) and measured by the phenolsulfuric acid technique of Montgomery (1957). Plasma glucose was determined by the ortho-toluidine method of Dubowski (1962) using 25-μl aliquots. Plasma lactic acid content was measured in 25-μl aliquots by a micromodification of the Sigma Chemical Co. (1968) technique.

RESULTS AND DISCUSSION

Throughout this study, swimming activity of all groups of fish in effluent or water was minimal. Fish in effluent appeared lethargic and less responsive to solution exchange or sampling disturbances than the control fish, particularly at 72 and 96 h. Mortalities of one and three fish were found in the groups of 12 fish sampled after exposure to effluent for 72 and 96 h respectively.

Liver glycogen — Liver glycogen levels were not altered during the first 12 h of effluent exposure (Fig. 1A). Longer exposures caused a progressive and marked decrease ($P < 0.001$ according to Student's t statistic) relative to control values. Liver glycogen reserves of fish exposed to effluent for 72 or 96 h decreased 86 and 80%, respectively, compared to values for corresponding control fish. Glycogen stores were constant for all control fish sampled after starvation for 2–6 days and did not differ ($P > 0.05$) from values for fish sampled directly from the stock tank.

Although numerous studies have examined the effects of exercise on glycogen energy reserves in fish, little information is available concerning the effects of other forms of stress on these reserves. Liver glycogen levels are depleted during acute hypoxia (Heath and Pritchard 1965) or physical disturbance of fish (Nakano and Tomlinson 1967). Fujiya (1961) found histological evidence for depleted liver glycogen reserves in fish held in cages in the proximity of a pulpmill outfall for 24 h. In a fashion similar to the present study, McLeay (1973) noted that liver glycogen levels in fish sampled after a 12-h exposure to pulpmill effluent were unchanged.

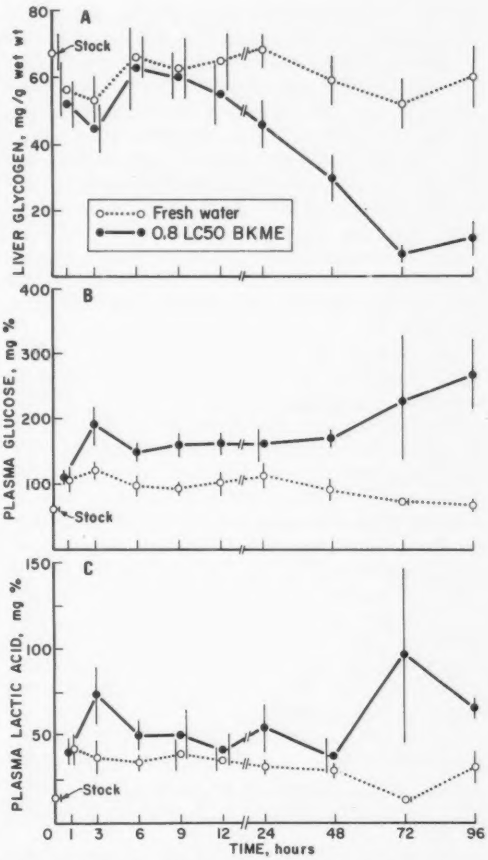

FIG. 1. Levels of A, liver glycogen; B, plasma glucose; and C, plasma lactate in fish held in 0.8 LC50 kraft mill effluent or water for 1–96 h. Values are means ± 2 SE.

Catecholamines deplete glycogen energy reserves in fish (Stimpson 1965; Nakano and Tomlinson 1967). On the other hand, glucocorticoids appear to stimulate the synthesis of liver glycogen (Swallow and Fleming 1970), presumably by stimulating gluconeogenesis. Thus, the marked glycogenolysis found in this study during acute exposure to BKME was probably caused by a stress-induced increase in circulating catecholamines.

Plasma glucose — Plasma glucose levels of control and effluent-exposed fish sampled at 1, 3, 6, 9, 12, 24, 48, 72, and 96 h following addition of 0.8 LC50 BKME are shown in Fig. 1B. Blood sugar levels in fish exposed to effluent for only 1 h did not differ from control values; mean values for all groups of fish held in effluent for periods from 3 to 96 h were elevated above corresponding control levels ($P < 0.001$). Between 24 and 96 h, mean values for blood sugar in effluent-exposed fish increased consistently whereas those of control fish declined (Fig. 1B). By 96 h, the mean blood sugar level was increased by 200 mg%, or 287% above the corresponding control value. Plasma glucose levels for all control samples were elevated above sugar levels in fish sampled directly from the stock tank.

Blood sugar levels are elevated in fish during acute exposure to a variety of environmental alterations considered as "stressful" (Chavin 1964; Nakano and Tomlinson 1967; Chavin and Young 1970; Narasimhan and Sundararaj 1971), including exposure to toxicants (Hunn 1972). Increased blood sugar levels have been found in juvenile coho salmon exposed to pulpmill effluent for 12 h (McLeay 1973). Stressful stimuli elicit rapid secretion of both glucocorticoids (Fagerlund 1967; Wedemeyer 1969) and catecholamines (Nakano and Tomlinson 1967) from the adrenal tissue of fish; both hormones produce a rapid hyperglycemia (McCormick and MacLeod 1925; Robertson et al. 1963; Young and Chavin 1965; Oguri and Nace 1966).

The increase in blood sugar levels of effluent-exposed fish with time reflects the "stress load" (Brett 1958) imposed, as evidenced by the 8 and 25% mortalities observed at 72 and 96 h, respectively.

The decline in blood sugar levels of control fish with time is thought to reflect their adaptation to transfer from the stock tank. Chavin (1964) showed that goldfish transferred from one aquarium to another required 4 days of adjustment to enable their blood sugar levels to return to normal.

Plasma lactate — Mean values for plasma lactic acid were elevated above those of fish in fresh water in all groups held in effluent for periods longer than 1 h (Fig. 1C). Increases were significant ($P < 0.001$) at 3, 6, 24, 72, and 96 h, but not at 9, 12, or 48 h. The reason for this decreased response at 9, 12, and 48 h is not apparent. Lactate levels for all groups of fish held in water except the 72-h sample were elevated ($P < 0.001$) above values for fish sampled directly from the stock tank.

As found with a high sublethal concentration of BKME in the present study, the lactic acid concentration in the blood of fish was elevated during acute exposure to a lethal concentration of an insecticide (Hunn 1972), and a heavy metal (Burton et al. 1972). McLeay and Brown (1974) found that blood and muscle lactate levels were elevated and blood pyruvate levels depressed in juvenile coho salmon exposed to kraft pulpmill effluent for 200 days. These changes, together with the increased blood sugar levels and decreased liver glycogen reserves found in the present study, are similar to those produced in fish receiving acute exposure to environmental hypoxia (Heath and Pritchard 1965; Burton et al. 1972). Such responses probably reflect tissue hypoxia. Davis (1973) reported that arterial oxygen tension in sockeye salmon (*Oncorhynchus nerka*) declined rapidly and remained depressed following up to 24-h exposure to BKME.

Although the exact cause of death of fish exposed to a lethal concentration of pulpmill effluent is unknown, it can be speculated from this study that mortalities are directly attributable to either tissue hypoxia, excessively high blood lactate levels, the complete exhaustion of available glycogen energy reserves or some combination of these factors. Pulpmill effluent has been shown to increase respiration rate and cough frequency (Walden et al. 1970; Howard 1973), and to reduce arterial oxygen tension in fish (Davis 1973). Therefore, there is no doubt that lethal or near lethal concentrations impair tissue respiration. High lactic acid levels have been associated with mortality of salmon (Black 1958; Parker and Black 1959). Parker et al. (1959) suggested that a lactate level of 125 mg% is lethal to salmon; levels of blood lactate found in fish exposed to pulpmill effluent were as high as 225 mg%. Miller et al. (1959) concluded that the exhaustion of glycogen energy reserves played a role in the deaths of planted trout. The 80% decrease in liver glycogen reserves of effluent-exposed fish at 72 or 96 h certainly reflects a severe depletion of carbohydrate stores.

Exercise–Rest Study

MATERIALS AND METHODS

Test fish — Coho salmon postalevin fry obtained from the Samish Hatchery in the spring of 1972 were held at low population density under conditions as described for the static study. Fish measured 7.7 ± 0.3 cm and weighed 5.6 ± 0.4 g at the time of the study.

Test effluent — A single batch of neutralized, filtered BKME with the following characteristics was used: 96-h LC50 value 45% (determined with 1 liter effluent/g fish per day), BOD_5 95 mg/liter, TOC 85 mg/liter, color 3750 APHA units, and sodium 265 mg/liter.

Experiments — A group of 10 fish from the stock tank was placed in each channel of a three-channel swim tunnel (Howard 1973) containing either 0.7 LC50 BKME or water, held constant at 12 ± 1 C. The fish were forced to swim against a velocity of one body length per second. Fish were sacrificed after 4, 8, and 12 h of exercise. Additional groups of fish were exercised for 12 h in effluent or water and then transferred to polyethylene tanks each containing 40 liters of either effluent or water. These fish were sacrificed after resting for 4, 8, or 12 h.

Sampling and analyses — The sampling and analytical procedures for plasma glucose and lactate, and liver glycogen were the same as those described in the static study. Additionally, a portion of muscle tissue weighing approximately 0.5 g was dissected from the epaxial musculature just below the dorsal fin of each fish. The tissue was then digested in 30% KOH for glycogen analysis according to the method of Montgomery (1957).

RESULTS AND DISCUSSION

Muscle glycogen — Muscle glycogen levels in fish sacrificed after 4, 8, or 12 h of exercise in effluent or water were similar ($P > 0.05$). Mean values for fish exercised in effluent for 12 h and sacrificed after resting in effluent for 4, 8, or 12 h, were decreased relative to those for fish resting in water (Fig. 2A) following 12-h exercise in effluent. These differences were significant ($P < 0.05$) at 4 and 12 h following exercise. Muscle glycogen levels were significantly lower for fish exercised and rested in effluent relative to those for fish exercised and rested in water at only 4 h of resting. Muscle glycogen reserves were depleted ($P < 0.001$ according to Student's t test) relative to stock values by 68 and 61%, respectively, after 4-h forced exercise in water or BKME (Fig. 2A). There was no return to stock muscle glycogen levels during 12 h of rest.

Strenuous exercise causes a marked and rapid depletion in muscle glycogen reserves in fish (Black et al. 1960, 1962) as in mammals (Drummond and Black 1960). In rainbow trout (*Salmo gairdneri*), muscle glycogen stores are reduced by 80–90% during 15 min of severe muscular activity (Black et al. 1961, 1962). The present results, i.e. 61–68% reduction in muscle glycogen reserves after 4-h exercise, with no recovery within 12-h resting, are in accord with these findings.

Liver glycogen — Liver glycogen levels were similar for fish sacrificed after 4-, 8-, or 12-h exercise in effluent or water ($P > 0.05$).

Liver glycogen reserves of fish exercised in effluent for 12 h and sacrificed after 4, 8, or 12 h of rest in effluent were decreased ($P < 0.001$) by 60–70% compared to corresponding control values, and by 55–65% from values for fish exercised in effluent for 12 h and rested in water for 4, 8, or 12 h (Fig. 2B).

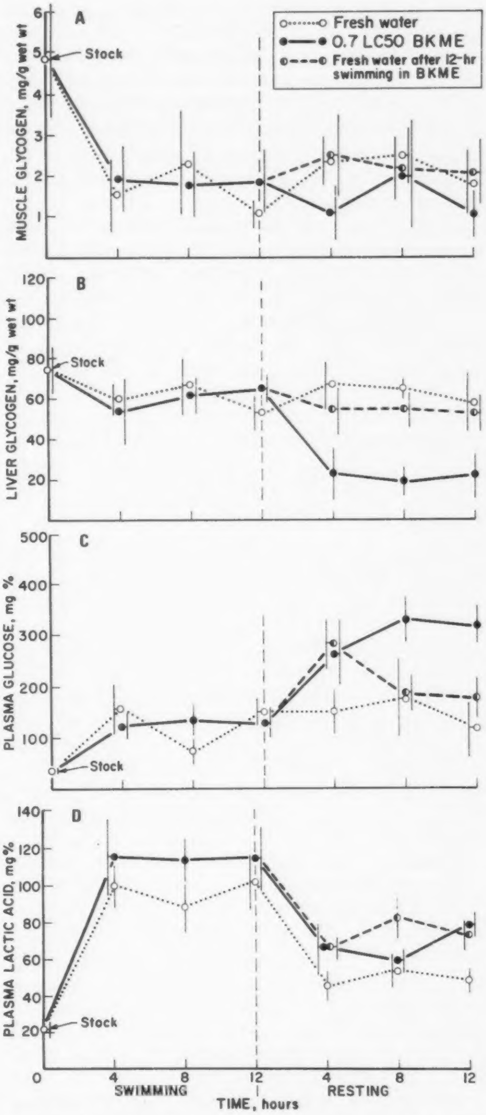

FIG. 2. Levels of A, muscle glycogen; B, liver glycogen; C, plasma glucose; and D, plasma lactate during and following moderate exercise in 0.7 LC50 kraft mill effluent or water for up to 12 h. Values are means ± 2 SE.

Mean values for all groups of exercised fish were somewhat decreased compared with the stock sample (Fig. 2B). Differences were significant ($P < 0.05$) for the following samples: control at 4 and 12 h and effluent-exposed at 4 and 8 h. Black et al. (1960, 1961) and Wendt (1965) found that liver glycogen reserves were not depleted during short periods of strenuous exercise, although values declined somewhat within a 24-h recovery period.

Twelve hours of exercise in effluent followed by a rest period of 4, 8, or 12 h in effluent resulted in a marked decrease in liver glycogen stores. This is consistent with the decrease found in unexercised fish held in effluent for 24–96 h in the static study. The lack of significant depletion of carbohydrate reserves in livers of fish exercised in effluent for 12 h and sacrificed after resting in water for 4, 8, or 12 h, implies that a significant response requires greater than 12-h exposure to effluent, and that any deleterious effects of a short (12-h) exposure to pulpmill effluent on liver glycogen stores are reversible.

Plasma glucose — Plasma glucose levels for fish exercised in effluent or water were similar ($P > 0.05$) at 4 and 12 h, but differed ($P < 0.05$) at 8 h (Fig. 2C).

During the resting phase in water, blood sugar levels of the controls were maintained at 120–175 mg%. In contrast, the fish resting in water after exercising in effluent showed a large increase ($P < 0.01$) in glucose at 4 h; by 8 h the levels had declined to those of the controls. Fish resting in effluent after exercising in effluent showed a similar elevation at 4 h, but the levels continued to increase to 331 and 316 mg% at 8 and 12 h, respectively (Fig. 2C), well above values for groups of fish resting in water ($P < 0.001$).

As in the static study, plasma glucose levels in all samples of fish in fresh water were elevated above stock values. This increase might reflect the stress imposed on the fish by the exercise, or simply reflects a response to transfer to a new environment (Chavin 1964). Black et al. (1960) found that blood glucose levels in rainbow trout were not altered during severe (15 min) or moderate (30 min) activity; however, values increased from 60 to 120 mg% during a 12-h recovery period.

Plasma glucose levels for fish sampled after exercising in effluent for 4, 8, or 12 h were similar to values found in unexercised fish held in effluent for 6–12 h during the static study (Fig. 1B). The apparent lack of a blood sugar response to pulpmill effluent during these exercise periods, unlike that due to effluent in the static study, was likely due to the elevated plasma glucose values in the fish exercising in water.

The elevation of blood sugar levels to greater than 300 mg% in fish resting in effluent for 8 or 12 h following exercise for 12 h in effluent, is indicative of a state of severe stress in these fish. As was the case in the static study, marked elevation of blood sugar levels coincided with a marked depletion of liver glycogen reserves. This extreme hyperglycemia might reflect an impairment of peripheral utilization of glucose recently mobilized from liver glycogen reserves by exposure to the effluent, or simply reflects rapid mobilization.

Plasma lactate — Mean plasma lactate values for fish exercised in effluent were consistently higher than controls, although differences were significant ($P < 0.01$) at 8 h only. Lactate levels of control fish resting in water for 4, 8, or 12 h following a 12-h exercise period, were decreased consistently from 100 mg% to 45–55 mg%. Mean values for fish resting in either effluent or water following exercise in effluent were consistently higher than corresponding controls and differed significantly ($P < 0.001$) except for fish resting in effluent for 8 h (Fig. 2D). Plasma lactate levels were similar for fish after 4 or 12 h of rest in either effluent or water following 12-h exercise in effluent.

Plasma lactic acid levels in fish during exercise in water or effluent for 4, 8, or 12 h were elevated to values 5–6 times those measured in fish sampled directly from the stock tank. Mean values for all groups of fish sampled after resting for 4–12 h remained elevated above stock values.

It is well documented that muscular exertion in fish is accompanied by a marked rise in blood lactate (Black et al. 1960, 1961; Beamish 1968). In the present study, it was found that during exercise a decrease in muscle glycogen accompanied the increase of plasma lactic acid. This supports the belief (Black et al. 1960, 1961) that muscle glycogen is the source of the lactate.

As found in the static study, mean values of plasma lactate for groups of fish exposed to pulpmill effluent were consistently elevated above control values. This increase indicates that the effluent has impaired aerobic metabolism; however, conclusive proof of the formation of an oxygen debt requires that the lactate:pyruvate ratio is elevated (Huckabee 1958). Since catecholamines can cause an increase in the blood lactate level of fish directly (Laarson 1973), this response to effluent might reflect a stress hormone-mediated response.

CONCLUSIONS

These experiments have demonstrated that acute exposure of young salmon to high sublethal concentrations of neutralized, filtered BKME causes marked alterations in a number of their carbohydrate metabolites, consistent with a stress response. Since plasma glucose and lactic acid levels were elevated in unexercised fish in BKME for 3 h or longer, it is thought that these responses might prove useful as a rapid method for measuring the toxicity of pulpmill effluents and possibly other toxicants. The plasma glucose response appears particularly suitable for measuring stressful levels of pollutants since the analytical procedure is simple and inexpensive. Further work is required to determine the sensitivity and reproducibility of these responses to a variety of toxicants under optimized test procedures.

Exercising fish in fresh water or in a high sublethal concentration of BKME for up to 12 h caused similar changes in their carbohydrate metabolism. Clear differences in levels of metabolites did not occur until the resting stage, at which time those fish exercised and rested in effluent showed elevated plasma glucose and lactic acid levels, and decreased liver and muscle glycogen values compared with fish exercised and rested in water. Although some of these changes were of a large magnitude, the complexity of test procedures may make utilization of these responses impractical as a routine sublethal bioassay.

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Density of the Commercial Spider Crab, *Chionoecetes opilio*, and Calibration of Effective Area Fished Per Trap Using Bottom Photography

R. J. MILLER

Department of the Environment, Fisheries and Marine Service, Newfoundland Biological Station,
St. John's, Nfld. A1C 5X1

MILLER, R. J. 1975. Density of the commercial spider crab, *Chionoecetes opilio*, and calibration of effective area fished per trap using bottom photography. J. Fish. Res. Board Can. 32: 761–768.

Bottom photography was found to be a more accurate method of censusing the commercial spider crab, *Chionoecetes opilio*, than trawling or sight counting from a submersible. Because of their highly aggregated distributions, estimating abundance of mature female crabs and immature crabs of both sexes was not practical by any method. The larger males ($\geq$40 mm carapace width), including those of commercial value, approached a random distribution, however; and abundance estimates for four study areas ranged from 5.2 to 9.2 crabs/500 m^2 and from 2.2 to 4.2 kg live weight/500 m^2. To permit results of exploratory fishing with traps to be converted to crab density and size of crab stocks available to a commercial fishery, a commercial crab trap was calibrated for "effective" area fished. The effective fishing area per trap averaged 4100 m^2 over four study areas.

MILLER, R. J. 1975. Density of the commercial spider crab, *Chionoecetes opilio*, and calibration of effective area fished per trap using bottom photography. J. Fish. Res. Board Can. 32: 761–768.

La photographie du fond, comme méthode de recensement du crabe des neiges, *Chionoecetes opilio*, s'avère plus précise que le chalutage ou le comptage à vue à bord d'un sous-marin. A cause des fortes agrégations qui caractérisent la distribution des femelles mûres et des immatures des deux sexes, aucune méthode n'est pratique pour en estimer l'abondance. Les grands mâles ($\geq$40 mm de largeur de carapace), y compris ceux de taille commerciale, ont toutefois une distribution à peu près aléatoire; les estimés d'abondance dans les quatre régions sous étude varient de 5.2 à 9.2 crabes/m^2 et de 2.2 à 4.2 kg de poids vif/500 m^2. Afin de convertir les résultats d'explorations de pêche aux casiers en densité et taille des stocks de crabes accessibles à la pêche commerciale, nous avons calibré un casier commercial par rapport à la superficie «effective» pêchée. La superficie de pêche effective par casier est de 4100 m^2 en moyenne dans les quatre régions étudiées.

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ONE of the first tasks in developing a new fishery is exploratory fishing to determine the size and location of the resource. The purpose of this study was to develop a method for obtaining unbiased density estimates for the spider crab, *Chionoecetes opilio*, that could be combined with data from exploratory fishing to calculate the size of the resource in Newfoundland.

Bottom photography was found to be the only suitable sampling method. Fishing success methods (e.g. DeLury 1947) require an active fishery and are not applicable for assessing virgin stocks. Even in fished areas in Newfoundland high fleet mobility and the short history of the fishery are obstacles to obtaining a sufficiently long time series of catch per unit effort. Although a tag has been developed for *C. opilio* that will be retained through a molt, its long-term retention and effects on mortality have yet to be tested (Dr. J. F. Karinen personal communication). If the tag does prove acceptable on these counts, the usual problems of emigration, immigration, and incomplete reporting of recaptures would complicate estimating population density from mark–recapture studies. Catches in traps, the usual commercial fishing gear for crabs, may provide relative densities, but calculation of absolute densities requires knowledge of the area fished by a trap. Both beam trawl collections and sight counts from a

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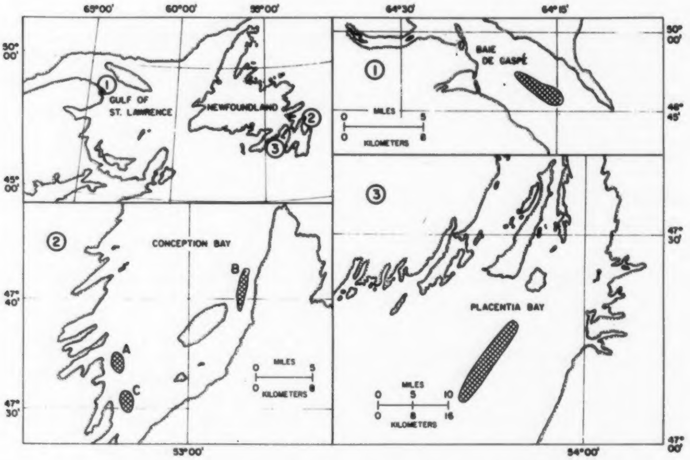

FIG. 1. Station locations within bays, indicated as hatched areas. The numbered locations on the small scale map, upper left, correspond to the numbers on the three large scale maps. Submersible operations were conducted in Baie de Gaspé and bottom photographs were taken in Placentia Bay and Conception Bay.

submersible were found in this study to underestimate density.

Materials and Methods

CRAB SIZE-CLASSES

Observations on the life history and fishery for *C. opilio* in Newfoundland provide the following useful size groupings for treating population data:

Carapace width (*mm*)	Characteristics
<40	males and females, immature only
40–69	males and females, mature or immature
≥70	mature males only
≥90	crabs retained in commercial traps
≥102	commercial sized crabs

Both sexes become sexually mature between 40 and 69 mm carapace width (personal observation). Females cease to molt after sexual maturity (Watson 1970) and rarely exceed 69 mm, whereas males reach a maximum size of 140 mm. In the Sea of Japan (Ito 1970) and the Gulf of St. Lawrence (Watson 1970), however, males reach 150 mm and females reach 90 mm. The 12-cm mesh used on traps in the Newfoundland fishery prevents escapement of nearly all crabs ⩾90 mm. Although there is no minimum legal size in Newfoundland, most processors have set a minimum acceptable size of 102 mm (4 inches).

OBSERVATIONS FROM A SUBMERSIBLE

Crab density and sizes were estimated by direct observation from a Perry PC 8 submersible in Baie de Gaspé in the western Gulf of St. Lawrence (Fig. 1, Table 1). By looking through the 1.2-m diameter front viewing port the number of crabs on a 1.7-m wide cruise track was counted. Each edge of the track was delineated by a rod attached to the submersible. As direct measurement of crabs was not possible, carapace width was estimated by visual comparison with a weighted meter stick suspended just off the bottom in front of the observer. The observer recorded his observations on magnetic tape.

Cruising speed of the submersible times duration of observations gave distance traveled. Speed was about 0.5 m/s (1 knot), as determined from a towed odometer wheel which produced an audible beep inside the submersible for each revolution (Caddy 1973). Beeps were recorded on tape and timed periodically to calculate speed (Caddy 1973).

BOTTOM PHOTOGRAPHY

An EG and G underwater camera with a 100-W-second strobe light was rigged to operate at bottom contact of a trigger weight (Carrothers 1967). The camera and light were mounted 1.4 m apart on a steel frame with the lens axis perpendicular to the bottom. The camera was lowered until the trigger weight contacted bottom, as indicated by deflection of a voltmeter visible to the winch operator, then hauled

TABLE 1. Study areas using a submersible or bottom photography.

Area	Collection date	Depth (*m*)	Bottom type	Bottom temperature (*C*)
Baie de Gaspé	Aug. 1971	76–94	Mud	1.5
Placentia Bay	Nov. 1971	195–225	Mud with rock outcrops	−1.1
Conception Bay				
Station A	Oct. 1972	230–270	Mud	−1.5
C	Oct. 1972	190–220	Mud with rock outcrops	−1.5
B	Oct. 1972	140–180	Sandy mud with rock outcrops	−1.5

up a few meters and lowered again after 15–30 s, the interval varying inversely with speed of the vessel. The vessel drifted at 0.15–0.5 m/s (0.3–1.0 knot) during photographic transects since it could not steam slowly enough to keep the wire angle close to vertical. The film was 30-m rolls of 35-mm Kodak TRI-X Pan, 400 ASA.

A 1.5 × 2.0 m grid of 20 × 20 cm squares was photographed in shallow water to calibrate the camera. Photographs were taken 2.8 m from the bottom giving a field of view of 2.4 × 1.4 = 3.4 m^2. This height was a compromise between maximum bottom coverage and adequate illumination plus precision in measurements of crab size. A chain, suspended from the camera frame and within the field of view, provided a check on control of the camera height off bottom. A photograph was not used if camera height was not within 20 cm of the calibrated distance. Crabs in the photos were counted and their carapace width measured by projecting the developed negatives on a screen or by viewing them under a dissecting microscope equipped with an ocular micrometer. Factors to convert the crab image to actual crab size were obtained by measuring the image of the squares of the calibration grid. Slightly different factors were used for different sectors of a photograph to correct for distortion and spherical aberration.

To calculate the variance of density estimates the photographs were separated into samples of either 500 or 100 m^2 of bottom coverage. Successive photographs taken at a station were included in a sample, e.g. the number of crabs in the first 147 photos (at 3.4 m^2/photo) at a station comprised the first 500-m^2 sample. Samples of 100 m^2 were used to calculate variances for an index to compare male and female aggregation. As females were found on only a small area of bottom, 100 m^2 samples were used to increase sample size.

Bottom photographs were first taken in Placentia Bay, and later in a more extensive survey at three stations in Conception Bay, all on the southeast coast of Newfoundland (Fig. 1, Table 1).

CRAB TRAPS

Although bottom photography gave acceptable accuracy in estimating *C. opilio* density, it is an expensive and tedious survey method. Therefore, I attempted to calibrate a commercial crab trap for effective fishing area. This was calculated as:

[1]
$$\begin{bmatrix}\text{effective fishing} \\ \text{area (m}^2\text{/trap)}\end{bmatrix} = \frac{\text{catch (crabs/trap)}}{\text{crab density (crabs/m}^2\text{)}}$$

where the crab density was estimated from the bottom photographs. If effective fishing area per trap is approximately constant it can be used to convert catch per trap, a relatively inexpensive statistic, to absolute crab density.

Effective fishing area per trap is not presumed to be the actual area of influence of a trap since the bait would likely attract only a fraction of the crabs present in a large area, presumably a decreasing fraction with increasing distance from the trap, rather than 100% in a smaller area as implied in the above calculation.

Traps were Japanese style, conical shaped with a top entrance, 1.2-m bottom diameter by 0.6 m high. Two to seven traps were set on a single groundline at 90-m intervals and baited with frozen herring. Traps were fished over 1 night except at Station A in Conception Bay where they were left for 2 nights because of bad weather.

Calculations of area fished per trap were based on all crabs $\geqslant$90-mm carapace width since the 12-cm mesh (stretch measure) on traps retained crabs down to at least that size. Size selection of traps was determined by fishing with 9.5- and 12-cm mesh traps.

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[1] This is a variation of the catch equation (catch/fishing effort = catchability coefficient × population size) where the catchability coefficient (m^2/trap) and population size (crabs/m^2) are expressed on an area rather than on the usual population basis (Morgan 1974).

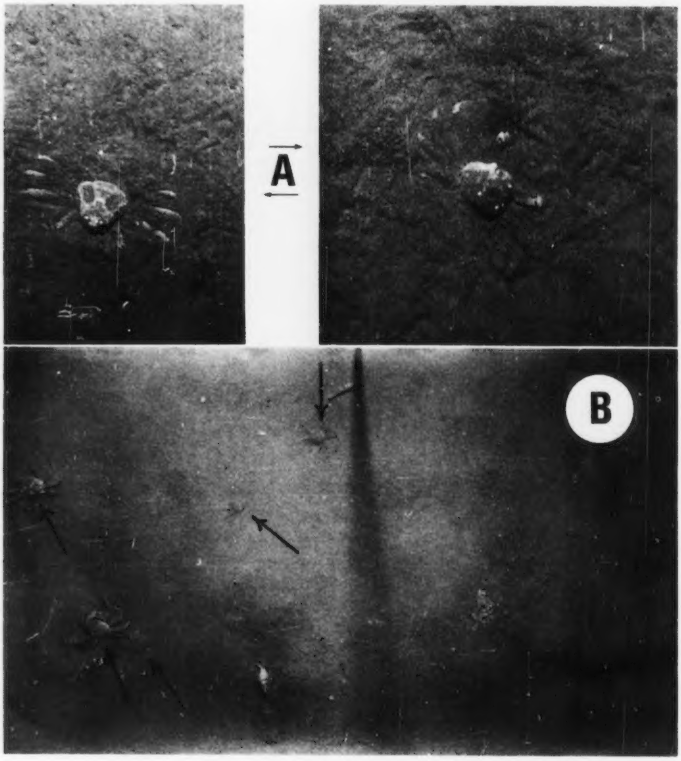

FIG. 2. A, Partially buried crabs at 160 m in Conception Bay, Nfld.; B, six female crabs on 3.4 m^2 of bottom at 220 m in Placentia Bay.

There was no difference in the number of crabs $\geqslant 90$ mm retained by the two mesh sizes ($P > .05$, $n = 59$), while traps with the smaller mesh retained more crabs <90 mm ($P < .05$, $n = 59$). Traps were set in each of the study areas.

BEAM TRAWL

Beam trawl sets were made on photographic stations to estimate sex ratios and to compare density estimates from the two methods. Sex of crabs could not be determined from photographs.

The trawl opening was 3.0 m wide by 0.6 m high, the mesh was 3.2 cm stretched measure and the foot of the net had a single chain attached. The ratio of cable length to water depth was 3:1 and the vessel was stopped after the cable was let out until the cable sank to an angle of 60° from the horizontal. Tows were 15–25 min at about 1 m/s (2 knots).

Results

VALIDATING PHOTOGRAPHIC METHODS

Shed exoskeletons and dead crabs might not be distinguishable from live crabs in photographs. Soft-shelled crabs were caught in traps in both Conception Bay and Baie de Gaspé, indicating that molting had occurred within the previous 3 mo (Watson 1971). However, no exoskeletons or dead crabs were recovered in beam trawl sets and no crabs seen from the submersible or in photographs were in positions (e.g. upside down) suggesting that they were not alive. In a subsequent study dead crabs placed in traps on the crab fishing grounds were dismembered and the carapace eaten through by amphipods within 7 days. This is likely the fate of shed exoskeletons as well.

Although nearly all crabs were found on soft bottom, they were seldom even partially buried, so they probably do not burrow out of sight. Crabs that were partly buried had both tops and sides of the carapace and parts of most legs exposed. The two crabs pictured in Fig. 2A were the most buried of 634 crabs photographed. By contrast, Watson (1969) observed from a submersible in shallower water (80–100 m) in the western Gulf of St. Lawrence that *C. opilio* was commonly partially buried in mud. It is possible that some small crabs were hidden among the occasional rock outcrops.

One roll of film provided a fortuitous check on the precision of crab measurements and the reaction of crabs to the strobe flash. For this film the vessel drift was so slow that there was considerable overlap in the bottom covered by successive photographs. In 15 separate sequences single crabs were photographed 2–9 times each. The greatest deviation of a measurement from the mean was 3 mm and the average coefficient of variation ($S \times 100/\bar{x}$) for the 15 sequences was 1.9%. Although I was concerned that crabs on a photographed area of bottom might have been frightened by the strobe flash of the previous photograph, photographs on this film were often less than 1 m apart and repeat photographs of the same crab indicated that the flash did not cause them to move away. This film was not used in estimating crab density.

AGGREGATION OF CRABS

Numbers and biomass of mature females and immature crabs of both sexes could not be estimated with reasonable precision because of their highly aggregated distribution.

A variance:mean ratio was used as an index of aggregation and was based on 30, 100-m^2 samples from two film transects in Placentia Bay. The composite catches of four beam trawl sets on these grounds are shown below. All crabs in the 40–69 mm range were females and 186 of 190 were sexually mature; thus all crabs in this size range in the photographs were also assumed to be female and predominantly mature. The absence of males of this size was unusual although they were always rare in trawl samples. Their location remains an unresolved problem.

Carapace width (*mm*)	Number of crabs	
	♂	♀
<40	2	1
40–69	0	190
≥70	51	0

Females (40–69 mm) were highly aggregated (Fig. 2B) whereas larger males (≥70 mm) approached a random distribution. The variance: mean ratio was 26 for females with a range of 0–66 crabs/100 m^2 and 0.93 for males with a range of 0–4 crabs/100 m^2. A ratio of one indicates a random spatial distribution, whereas a ratio greater than one indicates an aggregated or over-dispersed distribution (Cassie 1962).

Crabs smaller than 40 mm carapace width (immature males and females) were also aggregated. Although few were found in either trawl samples or photographs at any of the photographic stations, trawling in other areas revealed dense aggregations on rocky bottoms. In two such areas beam trawl tows sweeping a total of 5100 m^2 of bottom caught 144 crabs smaller than 40 mm, whereas on the photographic stations tows sweeping 71,000 m^2 yielded only 12 crabs smaller than 40 mm.

EFFECTIVE AREA FISHED PER TRAP

For four areas this statistic ranged from 2472 to 5293 m^2/trap with a mean of 4108 m^2/trap (Table 2). These results were based on a total of

TABLE 2. Calculation of effective area fished per crab trap using photography for estimates of crab density.

Station	Conception Bay			Placentia Bay
	A	B	C	
Area photographed (m^2)	6000	8000	6500	7000
No. crabs ≥90 mm/500 m^2 sample $\pm S_{\bar{x}}$	7.1±0.8	4.1±0.5	3.7±0.7	3.1[a]
No. traps fished	24	30	30	42
No. crabs ≥90 mm/trap $\pm S_{\bar{x}}$	35.1±3.1	43.4±3.3	33.1±3.0	26.0±3.4
Area (m^2) fihsed/trap	2472±341	5293±796	4473±914	4194[a]

[a] $S_{\bar{x}}$ (standard error of the mean) not calculated.

TABLE 3. Number of male crabs photographed and their biomass in grams live weight/m².

Carapace width (*mm*)	Conception Bay Stations						Placentia Bay	
	A		B		C			
	No.	g/m²	No.	g/m²	No.	g/m²	No.	g/m²
40–69	5[a]	0.1	6[a]	0.1	5[a]	0.1	10[a]	0.1
70–89	20	0.7	20	0.6	15	0.5	38	1.2
90–109	47	3.2	50	2.4	27	1.8	23	1.3
≥110[b]	38	4.3	15	1.2	21	2.2	20	2.1
Total	110	8.3	91	4.3	68	4.6	91	4.7
Commercial size (≥102)		5.6		2.0		3.3		2.5

[a] Number of males in this size-class reflects the sex ratios from trawl collections, 43% male in Conception Bay and 4% male in Placentia Bay.

[b] Maximum size 134 mm.

241 crabs ⩾90 mm carapace width photographed on 27,500 m² of bottom and 126 traps fished.

BIOMASS OF MALE CRABS

Male biomass ranged from 4.3 to 8.3 g/m² with most included in larger size-classes (Table 3). Biomass of commercial sized crabs was 68, 46, 72, and 53% of total biomass for the three Conception Bay stations and the Placentia Bay station, respectively. Placentia Bay had not been fished commercially and Conception Bay had supported a fishery for 2½ yr, but obviously not to the exclusion of large crabs.

The biomass estimates include only male crabs and only those larger than 39 mm carapace width since the patchy distribution of mature females and crabs smaller than 40 mm precludes estimating biomass or density with reasonable precision. However, mature females and small crabs were rare in both beam trawl samples and photographs at all three Conception Bay stations. Therefore, the biomass given for these stations approximates the total biomass present. Carapace widths (millimeters) were converted to live weight (grams) with the following regression equation (E. J. Sandeman unpublished data) based on measurements of 1900 crabs from 95 to 135 mm carapace width.

$$\text{Weight} = .000703\ \text{width}^{2.879}$$

CRAB DENSITY ESTIMATES FROM BEAM TRAWL AND SUBMERSIBLE

Beam trawl collections clearly underestimated crab density. In Placentia Bay density of crabs ⩾70 mm carapace width was (81/14 =) 5.8 crabs/500 m² when estimated from photographs and (113/70 =) 1.6 crabs/500 m² when estimated from nine beam trawl samples. The same comparison from Conception Bay (all three stations combined) was (253/41 =) 6.2 crabs/500 m² from photographs and (81/72 =) 1.1 crabs/500 m² from 12 beam trawl samples. Presumably crabs were able to avoid the towed net.

Large areas of bottom can be surveyed from a submersible, but the density of large crabs is apparently underestimated. The 11,000 m² surveyed from the submersible required only 3.5 h of observation time, whereas the 20,500 m² of bottom coverage with the camera in Conception Bay required 34 h of photography. Including equipment servicing and calibration of fields of view, vessel time was 2.5 days for the submersible and 14 days for the photography. Crabs could not be sexed with either method and precision of estimating sizes was probably better from photography. Crab measurements from photographs were made in the laboratory, whereas the observer made a quick decision on crab size from the moving submersible by first looking at a crab then at a meter stick. Independent observations by both me and another observer agreed that crabs larger than 80 mm width ran from the submersible whereas smaller crabs did not. Large crabs were most commonly seen only at the extremes of our visibility (2–4 m from the submersible) and running, whereas small crabs usually held their position and allowed the submersible to pass close by or over them. These observations plus an unusually high estimate of effective area fished per trap (see calculations below) suggest that the density of large crabs was underestimated.

Area observed from submersible (m²)	11,000
No. crabs ≥90 mm/500 m² sample $\pm S_{\bar{x}}$	1.4 ± 0.3
No. traps fished	16
No. crabs ≥90 mm/trap $\pm S_{\bar{x}}$	29.4 ± 4.1
Area (m²) fished/trap	10,400

TABLE 4. Example calculation of number of fishable crabs in a stock.

Depth (*fath*)	No. traps	Area (km^2)	Catch crabs/trap	Density crabs/km^2	Fishable crabs/km^2	Fishable crabs in depth zone
60–99	82	776	3.1	756	0	0
100–139	214	1049	21.3	5195	1536	1.6×10^6
140–179	48	49	25.5	6220	2561	0.13×10^6
≥180	12	15	12.5	3049	0	0
Total	356	1889				1.73×10^6

Discussion

Censusing crab populations by bottom photography was chosen because of the inadequacy of other methods. Beam trawl collections underestimated *C. opilio* density in this study as did collections from a modified otter trawl in the Gulf of St. Lawrence (Powles 1968). Samples from an Agassiz trawl underestimated total density of decapods in an epibenthic community (McIntyre 1956) as did a scallop dredge for density of *Cancer* sp. and *Pagurus* sp. (Wigley and Theroux 1970). Results given above showed that direct counts from a submersible was also an inadequate method for censusing large *C. opilio*.

The effective area fished per trap has limitations of both accuracy and precision, but offers the potential for making crude but inexpensive estimates of crab density. Catchability probably varies with type of bait (Perry 1969), trap fishing ("soak") time (Rothschild et al. 1970), temperature (K. Ishida personal communication, obtained commercial catches of *Chionoecetes bairdi* only above 1 C in the Bering Sea), stage of molt cycle (*C. opilio* in the laboratory did not feed for 1 wk after molting (Watson 1971)), trap design (Watson and Simpson 1969), and fullness of trap (although catches 50% greater than the greatest obtained in this study are common on some virgin fishing grounds). Also, the number of crabs photographed was not large, 43–85 crabs ⩾90 mm per station. Some of these factors no doubt contributed to the rather large spread among stations in estimates of effective area fished, 2472–5293 m^2/trap, as well as to within station variance (Table 2). In applying a value for catchability, data on seasonal changes in catchability would be helpful. In the commercial fishery in Conception Bay catch per trap typically drops by about 80% during May and June but does not vary substantially during the rest of the year. While it is possible that crabs move from traditional fishing areas in the bay during this period, exploratory fishing by fishermen and me in peripheral areas has not yielded higher catches.

For an Australian rock lobster population on coral and limestone reef habitat Morgan (1974) demonstrated that catchability varied with moon phase, salinity, temperature, stage of molt cycle, and presence of a predator in the trap. The effective area fished per trap (based on mark–recapture estimates of population density and catch per trap haul on 34 occasions) ranged from 40 to 196 m^2/trap, much lower values than the average 4100 m^2 found in this study. Rock lobster biomass was much higher, 12–75 g/m^2 (G. R. Morgan personal communication) vs. 2.0–5.6 g/m^2 for *C. opilio* in this study, and the reef habitat was much more irregular than the level bottom mud habitat of *C. opilio*.

An example will illustrate how effective area fished per crab trap may be used in estimating the size of a fishable stock (Table 4). An exploratory fishing survey conducted in 1969 and 1970 included most of Placentia Bay and spanned a depth range of 60–230 fath (110–420 m). The area (km^2) of the survey included in each of four depth strata and the mean catch of commercial-sized crabs (⩾102 mm) per trap in each stratum are given in Table 4. The density of commercial-sized crabs was calculated as follows:

$$\underset{(\text{no./km}^2)}{\text{Crab density}} = \frac{\text{crabs/trap}}{4100\ \text{m}^2/\text{trap}} \times \frac{10^6\text{m}^2}{\text{km}^2}$$

where the effective fishing area per trap is 4100 m^2 and the number of crabs per trap is taken from the survey. The approximate minimum catch for commercial feasibility is 15 crabs/trap, that is, fishermen do not continue to fish at lower average catches. Thus, crabs in the 60–99 and greater than 180-fath strata would yield below commercial catches and the other two strata would only be fished down to 15 crabs/trap. Subtracting the density of crabs yielding 15 crabs/trap (3659 crabs/km^2) from the stock density in each depth stratum gives the fishable crabs/km^2. The product of this value and the area in a stratum, summed over all strata, gives the number of fishable crabs in this virgin stock. In choosing strata one should attempt to ensure that a high percentage of the area in any one stratum yields catches either above or below the minimum commercial catch; otherwise the size of the fishable stock will be underestimated. For example, in Table 4 the catch deeper than 180 fath averaged less than 15 crabs/trap; however, if part of the stratum yielded catches greater than 15 crabs/trap, there would need to be a corresponding contribution to the fishable stock.

If crab growth data were available, this calculation could be extended to give annual recruitment to commercial size, that is, the number of crabs in the size range of the prerecruit molt times the fraction molting per year.

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Effects of pH on Toxicity of Antimycin to Fish

LEIF L. MARKING

Fish Control Laboratory, La Crosse, Wis. 54601, USA

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Detoxification of antimycin at pH 9.5 was caused by two factors. The piscicide was biologically unavailable at the high pH, and this unavailability was reversed by decreasing the pH of water solutions. Simultaneously antimycin detoxified with time, and the resulting loss in toxicity was irreversible. The toxicity of antimycin was related to the amount of un-ionized molecules; however, the dissociation curve resulting from the published pKa of 5.1 does not agree with the implied dissociation when based on toxicity. Toxicity of antimycin decreased gradually from pH 6.5 to 8.5 and abruptly from 8.5 to 9.5 with carp (*Cyprinus carpio*), green sunfish (*Lepomis cyanellus*), and bluegill (*Lepomis macrochirus*). As previously suggested, water hardness had little or no effect on toxicity.

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La détoxication de l'antimycine à un pH de 9.5 est due à deux facteurs. Le piscicide est biologiquement inaccessible à ce haut pH, et cette inaccessibilité est renversée par une diminution de pH des solutions aqueuses. Simultanément, l'antimycine se détoxifie avec le temps, et la perte de toxicité qui en résulte est irréversible. On constate que la toxicité de l'antimycine est liée à la quantité de molécules non ionisées; cependant, la courbe de dissociation résultant du pKa publié de 5.1 ne s'accorde pas avec la dissociation implicite, lorsque celle-ci est fondée sur la toxicité. La toxicité de l'antimycine diminue graduellement entre les pH 6.5 et 8.5, et abruptement entre 8.5 et 9.5 chez la carpe (*Cyprinus carpio*), le crapet vert (*Lepomis cyanellus*) et le crapet arlequin (*Lepomis macrochirus*). Comme nous l'avons déjà suggéré, la dureté de l'eau n'a que peu ou pas d'effet sur la toxicité.

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THIS study was designed to (1) determine whether detoxification of antimycin is a result of biological unavailability or chemical degradation that is implied in the literature, (2) determine whether loss in toxicity is reversible, (3) differentiate the influences of pH and water hardness on the acute toxicity to target and game species carp (*Cyprinus carpio*), green sunfish (*Lepomis cyanellus*), and bluegill (*Lepomis macrochirus*), and (4) identify the critical pH range at which antimycin becomes ineffective.

Some physical and chemical characteristics of water have been found to influence the toxicity of antimycin to fish. Walker et al. (1964) reported that antimycin degraded rapidly in alkaline water. Lee et al. (1971) investigated the influence of pH, hardness, and alkalinity on toxicity of antimycin to goldfish (*Carassius auratus*), a nongame species. They reported that pH was the only factor that influenced toxicity and that detoxification of antimycin was a function of pH at pH 9.0 and above. Marking and Dawson (1972) quantified the detoxification rate for antimycin in waters ranging from pH 6.0 to 10.0 and reported that the rate of detoxification was a function of pH. Hussain (1969) showed that degradation of antimycin followed consecutive first-order kinetics. None of the above investigators detected reversible toxicity of antimycin in water of high pH.

Materials and Methods

Fish were obtained from hatcheries and maintained by a fish culturist prior to tests (Hunn et al. 1968). Their weight at the time of the tests ranged from 0.4 to 1.5 g each. Test facilities and equipment conformed to those required for standardized laboratory toxicity tests (Lennon and Walker 1964). Technical grade antimycin was used in all tests.

Test waters were prepared by adding different quantities of salts to deionized water (Marking 1969). Total hardness as $CaCO_3$ was 10 mg/liter for very soft water, 44 mg/liter for soft water, and 170 mg/liter for hard water. The pH of test waters was

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TABLE 1. Buffering agents added (milliliters of agents per 15 liters of reconstituted water[a]) to obtain four pHs and to rebuffer soft water at pH 9.5 to a pH of 7.5.

pH	Agents	Strength	Very soft	Soft	Hard
6.5	NaOH	1N	4.0	5.0	1.0
	KH_2PO_4	1M	20.0	30.0	40.0
7.5	NaOH	1N	14.0	14.0	13.0
	KH_2PO_4	1M	20.0	20.0	20.0
8.5	NaOH	1N	3.5	6.5	6.0
	H_3BO_4	0.5M	20.0	40.0	30.0
9.5	NaOH	1N	9.5	11.0	15.0
	H_3BO_4	0.5M	20.0	20.0	17.0
7.5 (from 9.5)	KH_2PO_4	1M	—	13.5	—

[a] Hardness as milligrams per liter of $CaCO_3$ — very soft, 12; soft, 44; hard, 170.

TABLE 2. 96-h LC50s (micrograms per liter) and 95% confidence intervals in soft water at 12 C for green sunfish (*Lepomis cyanellus*) introduced 0–8 h after antimycin was introduced into solutions buffered to pH 9.5, rebuffered to pH 7.5, or unbuffered.

Time (*h*)	pH			
	9.5	7.5 (from 9.5)[a]	7.5 (from 9.5)[b]	7.5[c]
0	8.19 (6.55–10.2)	0.250 (0.217–0.289)	0.250 (0.217–0.289)	0.231 (0.209–0.255)
2	11.3 (10.4–12.2)	0.274 (0.222–0.388)	0.212 (0.171–0.262)	0.200 (0.164–0.243)
4	12.0 (11.3–12.7)	0.350 (0.318–0.386)	0.250 (0.204–0.307)	0.226 (0.193–0.265)
6	18.0 (15.2–21.3)	0.600 (0.492–0.732)	0.210 (0.170–0.260)	0.363 (0.292–0.451)
8	—	0.740 (0.574–0.955)	0.212 (0.158–0.285)	0.200 (0.150–0.267)

[a] Solutions rebuffered just before fish were introduced.
[b] Solutions rebuffered immediately after buffering to pH 9.5 (reference test).
[c] Soft water without buffering agents (reference test).

controlled with chemical buffers (Table 1) and checked daily. If the pH had changed by more than 0.2 unit, additional buffering agents were added as necessary. Temperature of test solutions was maintained at 12 C with a water bath.

Regressions were drawn from survival and mortality data for each experiment. Ten fish were exposed to each concentration, and at least nine concentrations were prepared for each test. The LC50s (concentrations producing 50% mortality) and 95% confidence intervals were computed according to the methods of Litchfield and Wilcoxon (1949). χ^2 tests were applied to each set of data to determine variability and acceptability.

Results

Although antimycin was significantly less toxic in water at pH 9.5 than at 7.5, the toxicity was restored when the pH was decreased to 7.5 by addition of buffers (Table 2 and Fig. 1). For instance, the 96-h LC50 for antimycin against green sunfish was 8.19 μg/liter at pH 9.5, but was 0.250 μg/liter in pH 9.5 solutions that were rebuffered to pH 7.5. The lines representing detoxification were nearly parallel for pH 9.5 solutions and those rebuffered to pH 7.5, but detoxification did not occur in reference tests at pH 7.5 for the short (0–8 h) aging periods (time before fish were introduced into toxicant solutions).

Unavailable antimycin at pH 9.5 became available when the pH 9.5 solutions were rebuffered to pH 7.5. In addition, detoxification occurred and corresponded to the loss in toxicity when toxicant solutions were aged 0 to 8 h before introducing the fish. Both mechanisms contributed to the detoxification of antimycin. The results of reference tests showed that antimycin in water rebuffered

TABLE 3. 96-h LC50s (micrograms per liter) and 95% confidence interval for fish in 12 C water of three hardnesses[a] and four pHs.

Species and pH	Very soft	Soft	Hard
	Carp		
6.5	0.100 (0.0744–0.134)	0.178 (0.134–0.236)	0.138 (0.107–0.178)
7.5	0.223 (0.177–0.280)	0.272 (0.217–0.340)	0.176 (0.130–0.238)
8.5	0.508 (0.372–0.694)	—	0.648 (0.468–0.863)
9.5	—	4.14 (3.29–5.22)	—
	Green sunfish		
6.5	0.111 (0.0598–0.206)	0.179 (0.151–0.211)	0.246 (0.218–0.278)
7.5	0.332 (0.243–0.454)	0.335 (0.262–0.429)	0.478 (0.400–0.571)
8.5	0.349 (0.189–0.645)	0.365 (0.292–0.456)	0.500 (0.440–0.568)
9.5	14.2 (11.5–17.6)	8.00 (6.70–9.55)	10.9 (9.17–13.0)
	Bluegill		
6.5	0.0919 (0.0654–0.129)	0.105 (0.0958–0.115)	0.107 (0.0844–0.136)
7.5	0.134 (0.110–0.164)	0.191 (0.165–0.221)	0.140 (0.100–0.196)
8.5	0.328 (0.235–0.457)	0.389 (0.330–0.459)	0.429 (0.340–0.541)
9.5	2.30 (1.82–2.90)	4.60 (3.63–5.83)	2.88 (2.14–3.88)

[a] Hardness as milligrams per liter of $CaCO_3$ — very soft, 12; soft, 44; hard, 170.

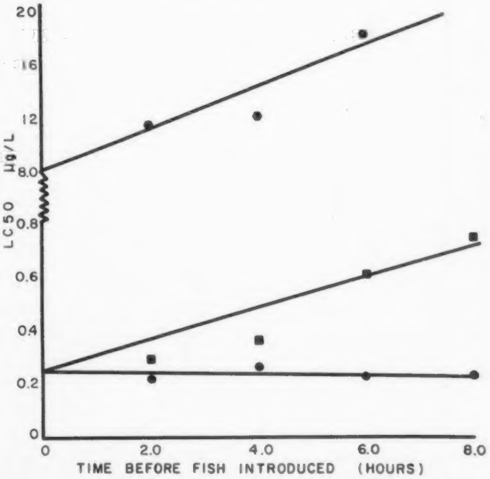

FIG. 1. Toxicity of antimycin in pH 9.5 water ⬢, in pH 9.5 water rebuffered to pH 7.5 ■, and in reference tests without buffers ● at 12 C.

to pH 7.5 immediately was about as toxic as antimycin in soft water containing no buffering agents (Table 2).

The toxicity of antimycin to fingerling carp, green sunfish, and bluegill was influenced considerably by the pH of water but little or not at all by water hardness (Table 3). These findings agree with those of Lee et al. using goldfish. In most instances, toxicity decreased significantly ($P = 0.05$) as pH increased from 6.5 to 9.5, but the greatest rate of decrease (by a factor as great as 40) was from pH 8.5 to 9.5 (Fig. 2). All three species responded similarly in the three water hardnesses at four pHs.

Discussion

The initial loss in toxicity of antimycin at pH 9.5 is caused mainly by the unavailability of the molecule, and this loss is supported by cell membrane permeability and chemical translocation theory (Loomis 1970; Jollow and Brodie 1972). Passage of drugs across gill membranes in fish is

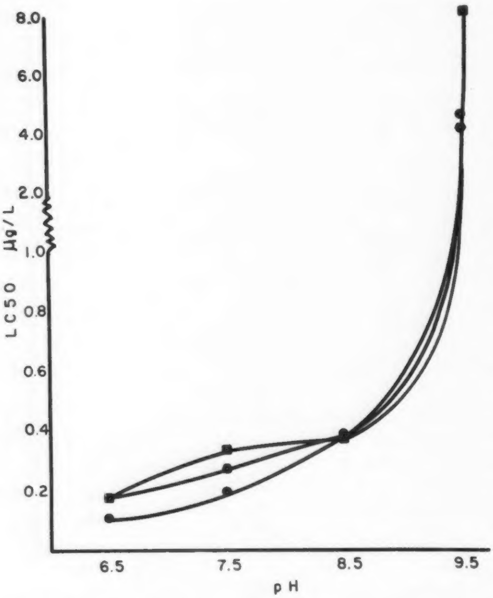

FIG. 2. Toxicity of antimycin to carp (*Cyprinus carpio*) ●, green sunfish (*Lepomis cyanellus*) ■, and bluegill (*Lepomis macrochirus*) ● in soft water at 12 C.

governed mainly by physical processes and is predictable from the pKa and lipid solubility of the drug (Hunn and Allen 1974). Antimycin, a weak acid, ionizes in alkaline solutions, and the ionized form is not expected to pass cell membranes as readily as the more lipid-soluble, un-ionized form that exists in acid media.

The pKa for antimycin was reported as 5.1 by van Tamelen et al. (1961). At that pH, the ratio of un-ionized to ionized is 1:1 and, theoretically, the un-ionized antimycin may be translocated readily across cell membranes. However, with a pKa of 5.1, the proportion of un-ionized antimycin becomes exceedingly low at pHs 7.5–9.5. The calculated percentage of un-ionized antimycin is 3.83 at pH 6.5, 0.39 at pH 7.5, 0.04 at pH 8.5, and 0.004 at pH 9.5. Accordingly, little antimycin is in the un-ionized form above pH 6.5. The availability of the un-ionized form does not correspond with fish toxicity data, since the significant loss in toxicity occurs above pH 8.5.

The difference in toxicity from pH 8.5 to 9.5 indicates that the ionization equilibrium for antimycin should be at a higher pH than 5.1, to correspond to the ionization theory. If the ionization curve for antimycin calculated for pKa 5.1 were shifted two pH units to the right, the difference in the toxicity at pHs 8.5–9.5 more closely corresponds to the ionization theory (Fig. 3). The change in toxicity is then nearly linear from pH 6.5 to pH 8.5 and corresponds with the toxicity data. The reported pKa of 5.1 for antimycin may be in error or there may be unrecognized additional pKa's.

A sudden change in the pH of treated waters could affect the efficacy of antimycin to target organisms or the safety to nontarget organisms. Various reports suggest that pH is responsible for ineffective treatments or overkills in experimental as well as management applications. Rabe and Wissmar (1969) reported that recommended concentrations of antimycin are higher than necessary for soft water and may result in a reduction of plankton.

Since pH influences the toxicity of antimycin, and the pH of natural water fluctuates, pH should be an important criterion in planning field applications. In the interests of economy and minimum concentrations needed, field applications should usually be planned to coincide with seasonal low pHs rather than for the convenience of administrative scheduling. However, when necessary, anti-

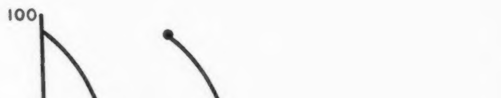

FIG. 3. Percent un-ionized antimycin in solution calculated from pKa's of 5.1 ■ and 7.1 ●.

mycin might be applied even though the pH is high, to take advantage of the rapid detoxification that occurs.

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Protein Instability in Minced Flesh from Fillets and Frames of Several Commercial Atlantic Fishes During Storage at −5 C[1]

J. R. DINGLE AND J. A. HINES

Department of the Environment, Fisheries and Marine Service, Halifax Laboratory, Halifax, N.S. B3J 2R3

DINGLE, J. R., AND J. A. HINES. 1975. Protein instability in minced flesh from fillets and frames of several commercial Atlantic fishes during storage at −5 C. J. Fish. Res. Board Can. 32: 775–783.

Minced flesh of Atlantic cod (*Gadus morhua*) and pollock (*Pollachius virens*), recovered by means of meat-separator machines from frames left after filleting operations, suffered a rapid loss of protein solubility during storage at -5 C. This was due to the presence of kidney tissue which caused the formation of dimethylamine and formaldehyde from the trimethylamine oxide of the muscle. The minced flesh of witch flounder (*Glyptocephalus cynoglossus*), American plaice (*Hippoglossoides platessoides*), and Atlantic mackerel (*Scomber scombrus*) was relatively stable when mixed with homogenates of their own kidney tissue, but cod kidney caused the same changes in gray sole as it did in minced cod flesh. The exclusion of gadoid kidney and blood from minced fish preparations is recommended.

DINGLE, J. R., AND J. A. HINES. 1975. Protein instability in minced flesh from fillets and frames of several commercial Atlantic fishes during storage at −5 C. J. Fish. Res. Board Can. 32: 775–783.

La chair hachée de morue (*Gadus morhua*) et de goberge (*Pollachius virens*), récupérée des squelettes à l'aide de machines à séparer les chairs après les opérations de filettage, subit une perte rapide de solubilité des protéines pendant l'entreposage à −5 C. Ceci est dû à la présence de tissu rénal entraînant la formation de diméthylamine et de formaldéhyde à partir de l'oxyde de triméthylamine du muscle. La chair hachée de plie grise (*Glyptocephalus cynoglossus*), de plie canadienne (*Hippoglossoides platessoides*) et de maquereau (*Scomber scombrus*) est relativement stable lorsque mélangée à des homogénats de leur propre tissu rénal, mais le rein de morue provoque les mêmes changements dans la plie grise que dans la chair hachée de morue. Nous recommandons l'exclusion du rein et du sang des gadoïdes des préparations de poisson haché.

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IT is well known that dimethylamine[2] and formaldehyde may be present in varying amounts in fish flesh, especially that of gadoid species (see review by Yamada 1968). Amounts of these substances sometimes increase during storage (0 to −10 C), and there is an accompanying decrease in the amount of protein that can be extracted by a salt solution (EPN) (Tokunaga 1970, 1974; Castell 1971; Dyer and Hiltz 1974). There is evidence that the DMA and FA are formed by an enzymic degradation of the TMAO normally found in most marine fish muscle, and that this activity occurs in several visceral organs, and also in the dark muscle of Alaska pollock and red hake (e.g. Amano and Yamada 1964; Tokunaga 1964, 1974; Castell 1971; Dyer and Hiltz 1974).

Castell (1971) and Castell et al. (1973) showed that the loss of extractability could be related to the amount of FA formed in storage. Dyer (1973) has postulated that a reaction between FA and protein is the major mechanism of protein aggregation in gadoid species, particularly in Alaska pollock and red hake, where its effect far surpasses that of other proposed mechanisms such as salting out or reaction with fatty acids.

The decrease in EPN in fish muscle is related to increased toughness when cooked, and a consequent decline in acceptability by taste panels

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[1] Some of the results reported here were used in preparing the paper, "Frozen Storage Characteristics of Minced Fish," by W. J. Dyer, presented at the Second Technical Seminar on Mechanical Recovery and Utilization of Fish Flesh, Boston, Mass., June 12–13, 1974.

[2] The following abbreviations are used: TMAO, trimethylamine oxide; TMA, trimethylamine; DMA, dimethylamine; FA, formaldehyde; EPN, extractable protein nitrogen; FFA, free fatty acids; TBA, thiobarbituric acid; TCA trichloracetic acid; EDTA, ethylene-diaminetetraacetic acid.

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(Dyer 1951). At the same time, there is a loss of water-holding capacity of the muscle, which in turn can lead to difficulties in secondary processing, such as the preparation of fish sticks.

There is an increasing commercial use of meat-separating machines ("deboners") for recovery of edible flesh from the "frames" (i.e. skeletons) remaining after removal of the fillets from beheaded and gutted fish. The same technique is also being applied to presently underutilized fish without filleting them, especially smaller species. Tokunaga (1964) and Babbitt et al. (1972) have reported, however, that the formation of DMA and FA occurs more rapidly in minced flesh than in intact fillets of Alaska pollock and Pacific hake. Little else is known about the frozen storage stability of minced fish flesh, patricularly from frames, so we have investigated this for several fishes of commercial importance in the Northwest Atlantic fishery. To save time, we chose −5 C as the storage temperature, because it permits chemical changes to occur at convenient rates, but stops the growth of psychrophilic bacteria (Castell 1971). Although normal commercial storage temperatures are lower, it is also unfortunately true at present that such a high temperature is sometimes reached, particularly in transport and in retail stores. We also measured lipid changes in an attempt to assess their importance relative to other possible mechanisms of protein denaturation.

Materials and Methods

The fish studied included Atlantic cod (*Gadus morhua*), pollock (*Pollachius virens*), witch flounder (*Glyptocephalus cynoglossus*), American plaice (*Hippoglossoides platessoides*), and Atlantic mackerel (*Scomber scombrus*). They were obtained from the commercial fishery, and the time after catching varied from about 4 h (mackerel) to 10 days. Following commercial practice, the cod and pollock were gutted before icing, but the flounder, plaice, and mackerel were not. The fish were filleted after landing, and in some cases the skins were removed from the fillets.

Mincing was accomplished by passing the fillets through a Bibun meat-separator (Models DX-15 and SDX-16), operated at near maximum belt tension and fitted with a drum having 5-mm holes. Unskinned fillets were placed with the skin against the belt; most of the subcutaneous brownish muscle, but very little skin, passed through the drum.

Cod "frames" remaining after removing the fillets from the gutted and beheaded fish, were also passed through the separator. The frames contained the swim bladder and the kidney tissue which are not removed with the other viscera in commercial practice. These tissues passed through the drum along with the muscle tissue. In one experiment, some of the material was washed by stirring for 15 min with 5 parts of water at 4–5 C, and then the solids were recovered in a Bird decanter centrifuge.

In certain experiments, kidney tissue was removed and homogenized with 2–3 parts of ice-water, and then mixed thoroughly by hand with the minced muscle (7.5–20 g/kg of mince). In one experiment with cod flesh, 4.5 kg of minced fillet was similarly mixed with 75 ml of fresh blood drained from the severed tail of an aquarium-held cod killed by a blow on the head.

The prepared materials were packed in 454-g (1 lb) wax-coated cardboard boxes and quick-frozen in a plate freezer at −40 C. Two samples for controls were repackaged under vacuum in nylon–saran–polyethylene triple-laminated bags, and stored at −40 C. The other samples (in their boxes) were wrapped in polyethylene bags and stored in a cabinet controlled at −5 ± 0.5 C. One sample of each material was taken at intervals for analyses; the "zero time" samples were taken 18 h after being placed in the −5 C cabinet.

Moisture content was determined by drying samples for 18 h at 105 C. The water contained in the samples used in the various extractions was considered as part of the volume of the extracting solution for calculating the total amount of the component being assayed.

Extractable protein nitrogen was determined by applying the biuret procedure (Snow 1950a) to the centrifuged supernate of an extract made by blending 22.5 g of sample with 428 ml of salt solution (5% NaCl in 0.003 M $NaHCO_3$) (Dyer et al. 1950). Correction was made for turbidity, and a factor, $k = 0.40$, was used in calculating protein nitrogen.

Extracts made by blending 50 g of sample in 100 ml of 6% perchloric acid, followed by centrifugation and neutralization with 15 M KOH, were used for the determination of DMA, by the Cu-dithiocarbamate method (Dyer and Mounsey 1945); TMA, by the picric acid procedure of Dyer (1945) (see also Castell et al. 1974), modified by using KOH as the alkalizing reagent (Tozawa et al. 1971); and FA, by the procedure of Nash (1953), modified by Cochin and Axelrod (1959) (see also Castell et al. 1973). TMAO was measured according to Dyer et al. (1952).

Lipids in 100-g samples were extracted according to the method of Bligh and Dyer (1959). Free fatty acids were measured in the extracts by the titration procedure of Bligh and Scott (1966), and expressed as milligrams per 100 g of sample, assuming an average molecular weight of 300.

For the thiobarbituric acid value, the procedure of Vyncke (1970) was applied to extracts made by blending 15 g of sample with 30 ml of 7.5% trichloracetic acid containing 0.1% each of propyl gallate and EDTA. It was found that a cloudiness developed during the heating stage if the ratio of extracting solution to fish was as high as 5:1, presumably because the TCA in the extracting solution was interfering (D. I. Hiltz and D. W. Lemon personal communication).

To measure water retention, an adaptation of the of the method of Wierbicki (1957) was used. A

250-ml plastic bottle, with the top cut off and 2-mm holes drilled in the bottom, was supported in a stainless steel centrifuge cup on a ring about 2.6 cm in height cut from plastic pipe. Samples of 100 g were allowed to thaw at 25 C in the cups, and the amount of "drip" collected was weighed. The cups and samples were then centrifuged at 650 × *g* for 2 min, and the total amount of liquid was weighed and designated "expressible fluid." Fresh or undenatured muscle tended to pass through the holes or to plug them and prevent the passage of liquid, but with deterioration of the muscle the performance of the apparatus greatly improved. Fine screens and filter paper were useless with uncooked fish.

Measurements of pH were made on homogenates of 1–2 g of samples in 20 ml of 0.005 M Na iodoacetate.

Results and Discussion

The changes in EPN and DMA in the material obtained by passing beheaded cod frames through the deboner are shown in Fig. 1. EPN reached a minimum level after 10 days at −5 C, but the DMA continued to increase for at least 17 days. DMA production in the washed material was much less than in the unwashed mince, as would be expected if the substrate, TMAO, and perhaps a causative enzyme and cofactor or both together with much of the water-soluble protein as well, had been largely removed by the washing (Tamoto 1971). The formaldehyde found in the unwashed mince was only about 30% of the DMA, probably because reaction with the protein in the mince made part of it unavailable for assay (Castell and Smith 1973). For this reason, FA was not measured in subsequent experiments, and production of DMA was used as a measure of the progress of the reaction. Initial loss of EPN (0.53 g/100 g at 0 days) in the washed material during equilibration at −5 C was greater than the corresponding loss (0.42 g/100 g) in the unwashed material, even though the DMA produced was only about 10% of that present in the unwashed product. We suspect that the reduction of the electrolyte concentration caused by washing made the actomyosin proteins more sensitive to denaturation by freezing (Snow 1950b). TMA in the unwashed mince was about 1.1 mmol/100 g (15 mg N/100 g), but this level did not change during storage. The washed mince contained only 0.25 mmol TMA/100 g throughout the storage period of 17 days. The pH of the unwashed mince increased during storage from 7.10 to 7.45, reflecting the rather high and increasing amounts of total free amines (TMA and DMA); in the washed mince it was 6.7 ± 0.2 during storage.

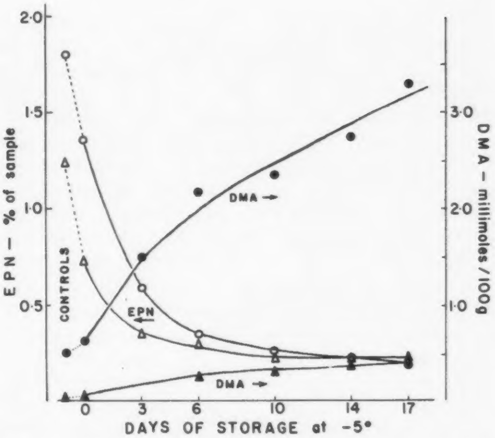

FIG. 1. Changes in extractable protein nitrogen and dimethylamine in minced flesh from cod frames during storage at −5 C. Open symbols, EPN; solid symbols, DMA. △ and ▲, flesh washed before freezing; ○ and ●, not washed.

In another experiment, fillets cut from gutted cod (2 days on ice) were skinned and passed through the deboner, and part of the material obtained was mixed with fresh cod blood (16 ml/kg). The frames remaining after filleting were beheaded and deboned as well. Results of EPN and DMA assays are given in Fig. 2. The results with the material from the frames were dramatic like those in the first experiment. With the minced fillet, however, the EPN remained stationary for about 10 days and then declined slowly to about 72% of its initial value at 29 days storage; accumulation of DMA in this time was only 0.24 mmol/100 g. The addition of blood to the minced fillet caused the protein extractability to decrease more rapidly during storage, and also caused a small but consistent increase in the rate of production of DMA. These changes were much less pronounced than those observed in the material from the frames.

Changes in the water retention of the materials are shown in Fig. 3. The amount of free drip from the minced fillet remained fairly constant at 4–5%, but that from the deboned frame material declined from an initial value of about 11% to 0 with 14 days. At the same time, the amounts of liquid removed from the latter material by centrifuging increased from about 20% to about 46% in 21 days, despite the lack of any free drip in the later stages, while that from the minced fillets increased from 10 to 29%. The behavior of the minced fillet plus blood mixture again appeared to be intermediate. This tendency of the free drip

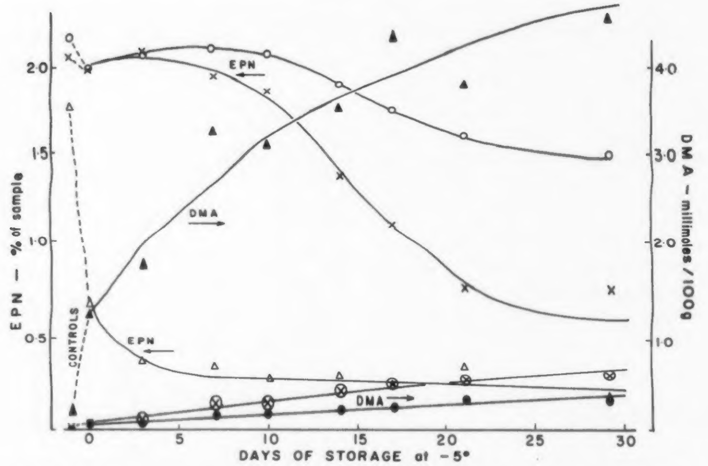

FIG. 2. Changes in extractable protein nitrogen and dimethylamine in minced flesh of cod during storage at −5 C. △ and ▲, flesh from deboning of cod frames; ○ and ●, minced fillet; × and ⊗, minced fillet plus blood. Open symbols, EPN; solid symbols, DMA.

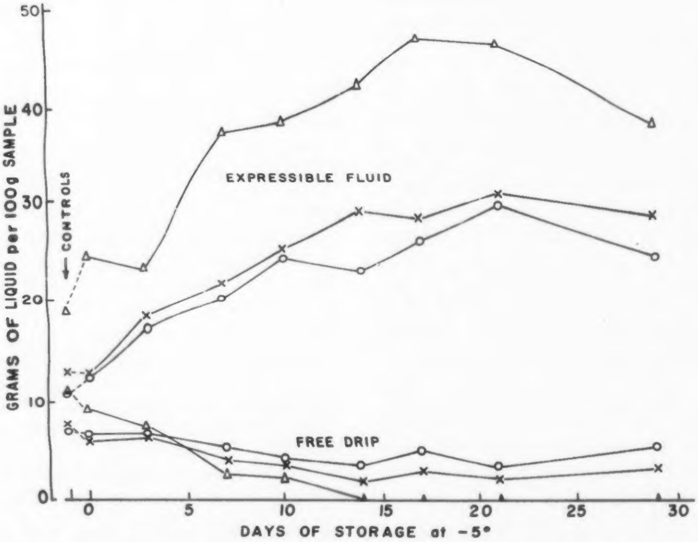

FIG. 3. Changes in free drip and expressible fluid of minced cod flesh during storage at −5 C. ○, minced fillet; ×, minced fillet plus blood; △, flesh from deboning of frames.

to decline during frozen storage, while the expressible liquid increased, was also noticed in other experiments in the present study in which the measurements were made. TMA concentrations in all materials were low (0.01–0.06 mmol/100 g) and remained so during storage. The pH of the frame material remained near 7.3, and that of the other minces near 6.8.

The foregoing results suggest that the rapid changes observed during storage of the minced material from cod frames might have been catalyzed by blood remaining in the frames, but it seemed possible that the kidney tissue also played a part. Several tissues of gadoid fishes, particularly the pyloric caeca, have been shown to contain DMA and FA (e.g. Amano et al. 1963), but there seems to have been no similar study of kidney tissue. Comparison of the storage behavior of minced cod fillet, and of the same material mixed with a homogenate of cod kidney (20 g kidney/kg mince) showed that the kidney tissue is indeed very active in causing formation of DMA (Fig. 4). In this experiment, the fillet mince deteriorated more rapidly than the corresponding material in the previous experiments. These fillets had been passed through the deboner with the skin on, whereas the earlier ones had first been skinned by hand. It is possible that this resulted in the inclusion in the mince in this experiment of a larger proportion of the subcutaneous dark muscle tissue, which in the case of red hake (Castell 1971) and Alaska pollock (Tokunaga 1974) has been implicated as one cause of DMA and FA formation.

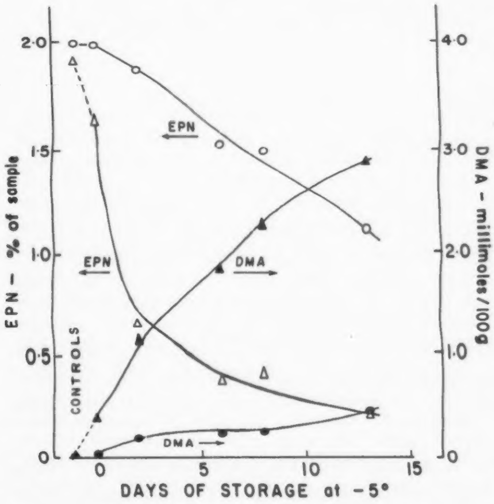

FIG. 4. Effect of a homogenate of cod kidney on changes in extractable protein nitrogen and dimethylamine in minced cod flesh stored at −5 C. ○ and ●, minced fillet; △ and ▲, minced fillet plus kidney homogenate; open symbols EPN; solid symbols, DMA.

The behavior of pollock muscle in storage at −5 C was very similar to that of cod (Fig. 5). In addition to minced fillets with and without pollock kidney homogenate, portions of intact fillets were frozen and stored in 454-g (1 lb) blocks. There did not appear to be any difference in protein stability or in the production of DMA between the minced and the intact muscle. In the presence of kidney tissue, the protein of the mince was rapidly denatured and DMA rapidly formed, the latter reaching a plateau of about 3.6 mmol/100 g within 7 days.

Loss of EPN from minced flesh of witch flounder, mackerel, and American plaice was slow compared with the rates found with cod and pollock, and the presence of the respective kidney tissue had no apparent effect (Fig. 6). Production of DMA was very small or negligible in all samples. TMA was also low and did not change during storage; the highest value found was 0.54 mmol/100 g in the witch flounder controls, and this was probably caused by bacterial action during the 10 days these fish were in ice before being landed. All of the fish contained an adequate amount of TMAO (3–4 mmol/100 g) (40–65 mg N/100 g) for the production of FA and denaturation of protein if the necessary conditions had been present. The pHs of the two flatfish minces were in the range 6.7–7.0, but in the mackerel the pH range was 5.9–6.0. In the latter case, it is conceivable that the low pH may have inhibited the formation of DMA and FA, since Tokunaga (1964) found a rather sharp pH maximum at 7.2 for this reaction in frozen aqueous extracts of Alaska pollock muscle.

To test the potential vulnerability of such apparently stable fish muscle, 6.4 kg of minced fillet from a second group of witch flounders was mixed with a homogenate of 85 g of cod kidney, frozen, and stored at −5 C. There was a strong production of DMA and a sharp drop in EPN in the mixture within 10 days (Fig. 7), while in the samples without cod kidney there was no change in EPN and no production of DMA. Thus, witch flounder kidney is unable to produce DMA and FA from TMAO in significant amounts, but witch flounder muscle will be damaged if mixed with a tissue possessing this activity.

It is noteworthy in the foregoing experiments that in the presence of active kidney tissue the

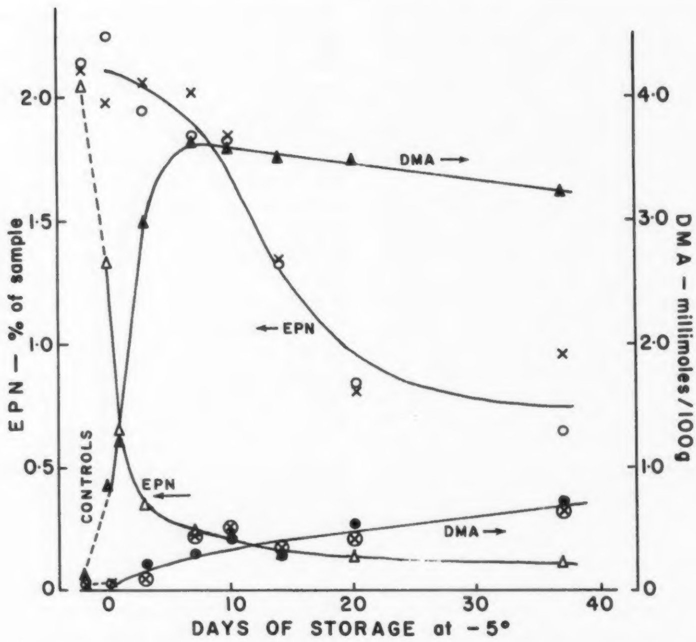

FIG. 5. Effect of a homogenate of pollock kidney on changes in extractable protein nitrogen and dimethylamine in minced pollock flesh stored at −5 C. ○ and ●, minced fillets; △ and ▲, minced fillets plus kidney homogenate; × and ⊗, intact fillets.

EPN values tended to fall below 23% of the initial value for the flesh, which is the proportion of water-soluble proteins ("albumins") in cod muscle (Dyer et al. 1957). It seems likely that the strong initial decrease in EPN was due to attack of the structural proteins (actomyosin) by the FA, and that loss of the presumably less sensitive albumins is represented by the much smaller slopes of the remainders of the curves.

In all the samples examined, the amount of free fatty acids in the lipid extracts increased with storage time (Fig. 8A and B). The presence of kidney tissue appeared to have no effect, except in the case of pollock in which the production of FFA was somewhat lower than in the plain mince or fillets. The largest amount of FFA was formed in mackerel (about 700 mg FFA/100 g of meat after 15 days at −5 C; total lipids 8–10%). In the other species, the FFA amounted to 250–320 mg/100 g (28–45% of the total lipids) at the end of their storage periods. TBA values were measured only in mackerel and in pollock fillet minces stored at −5 C, and were 1.2 μmoles of malonaldehyde/100 g in mackerel after 35 days and 0.26 μmoles/g in pollock after 59 days.

There has been much work and discussion on the role played by free fatty acids and aldehydes formed by changes in the lipids of fish flesh in frozen storage, and these may well have been responsible for the slow denaturation of the proteins observed in the present work in the absence of DMA and FA formation in both gadoid and nongadoid species. In the presence of gadoid kidney tissue, however, any effect of FFA becomes negligible because of the strong production of highly active formaldehyde.

These experiments confirm the reports (Tokunaga 1970, 1974; Castell 1971; Dyer and Hiltz 1974) that the flesh of gadoid fishes tends to be less stable than that of other species in frozen storage. They have also disclosed that the FA-forming activity of kidney and blood will cause much more rapid deterioration of the flesh if these are present as a result of using deboning

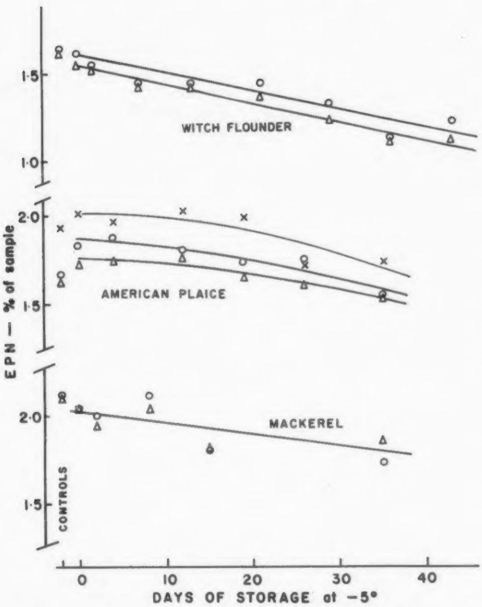

FIG. 6. Changes in extractable protein nitrogen in minced flesh of witch flounder, American plaice, and mackerel, in the presence and absence of homogenates of the respective kidney tissues during storage at −5 C. ○, minced fillets; △, minced fillets plus kidney tissue; ×, intact fillets (American plaice only).

machines to recover edible flesh from cod and pollock. The available data do not permit a direct comparison of the effects of blood and kidney tissue, but an examination of Fig. 2 and 4 suggests that the kidney tissue (which would contain some blood) is the more active one.

It will therefore be necessary to remove the kidney and blood from the gadoid species before deboning, but in cod and pollock, at least, this will be difficult. In these fish the kidney tissue adheres to the backbone, and is separated from the body cavity by the tough swim bladder which is fastened securely to bony extensions of the vertebrae. To the rear of the body cavity, these bones form arches that shield the thicker, rear portion of the kidney. Consequently, it may be necessary to cut away the entire spinal column before deboning. An alternative procedure, which would apply as well to those fish such as red hake in whose flesh FA is formed even in the absence of kidney or blood (Castell 1971), would be to remove the TMAO substrate by washing, but in this case it will probably prove necessary to use weak solutions of salt or other additives rather than plain water. This might protect the structural proteins from another type of freeze-denaturation. Effective techniques of this nature are now used in the production of frozen surimi in Japan (Takeda et al. 1971; Tanikawa 1971), and King (1973) has discussed the washing of minced fish. The "albumins" also removed by the washing could be recovered by one of several known methods.

The kidney tissues of mackerel, witch flounder, and American plaice do not cause deterioration of the flesh, and in any case are more readily accessible than in cod or pollock. They would be easier to remove if desired in order to improve the color and flavor of the deboned fish.

It has been suggested (Miyauchi and Steinberg 1970) that minced flesh from two or more species of fish might be mixed to adjust texture or flavor. We carried out an experiment in which minced muscle of pollock and witch flounder were mixed, and the rather inconclusive results indicated that in this case the denaturation of the proteins at −5 C was additive. Much more work on the storage stability of mixed minced flesh is required,

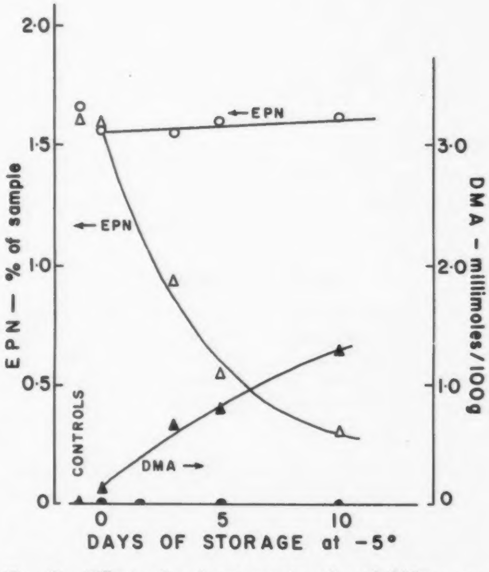

FIG. 7. Effect of a homogenate of cod kidney on extractable protein nitrogen and dimethylamine in minced witch flounder fillets during storage at −5 C. ○ and ●, minced fillets; △ and ▲, minced witch flounder fillets plus cod kidney homogenate.

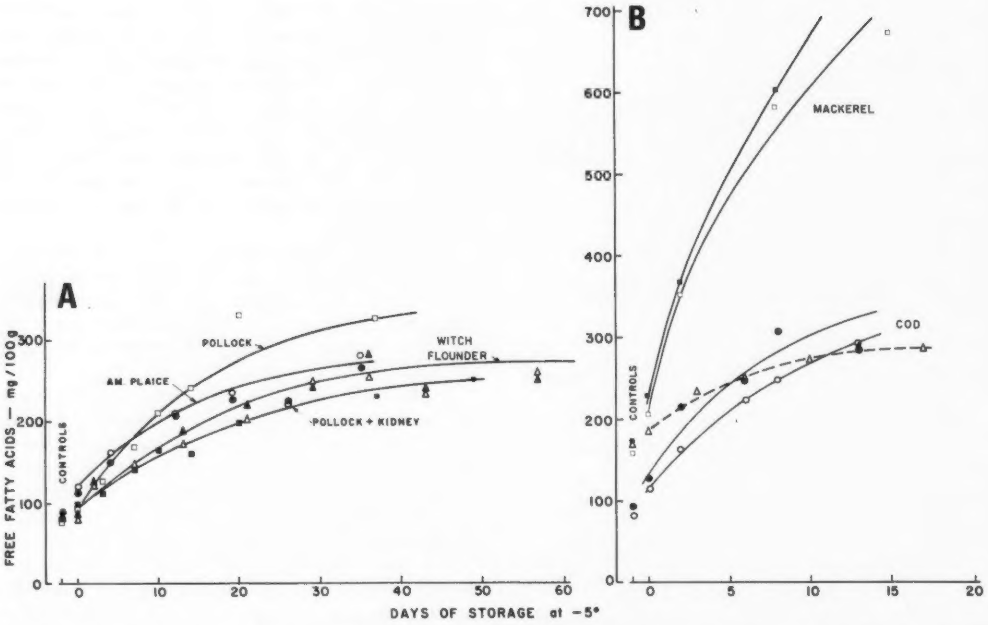

FIG. 8. A, Production of free fatty acids in minced fillets of various fishes, and the effect of homogenates of their respective kidney tissues, during storage at −5 C. Open symbols, minced fillets; solid symbols, minced fillets plus kidney homogenates. ○ and ●, American plaice; △ and ▲, witch flounder; □ and ■, pollock; B, production of free fatty acids in minced flesh of cod and mackerel, and the effect of homogenates of their respective kidney tissues, during storage at −5 C. Open symbols (○ and □ only), minced fillets; solid symbols, minced fillets plus kidney homogenates; △, flesh from deboning of cod frames.

but in the meantime it appears inadvisable to mix flesh from gadoid fishes with that of other species, at least until some means of reducing the production of FA in the former is devised.

Acknowledgments

The preparation of the minced flesh was carried out in the Laboratory pilot plant under the supervision of R. Legendre. We also acknowledge the valuable assistance of W. Robson and W. D. Aubut who carried out many of the analyses.

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Effect of DDT on Discriminating Ability of Rainbow Trout (*Salmo gairdneri*)

PATRICIA G. MCNICHOLL AND W. C. MACKAY[1]

Department of Zoology, University of Alberta, Edmonton, Alta. T6G 2E1

MCNICHOLL, P. G., AND W. C. MACKAY. 1975. Effect of DDT on discriminating ability of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 32: 785–788.

Rainbow trout (*Salmo gairdneri*) trained to discriminate between a bright (723 lx) and a dim (22 lx) light were tested to determine the minimum difference in light intensity they could distinguish before and 71 h after being given DDT orally at 0, 30, 50, and 80% of the LD50 dose. Discriminating ability decreased linearly with increased dose, while performance and retention were unaffected.

MCNICHOLL, P. G., AND W. C. MACKAY. 1975. Effect of DDT on discriminating ability of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 32: 785–788.

Des truites arc-en-ciel (*Salmo gairdneri*) ayant appris à distinguer un éclairage brillant (723 lx) d'un éclairage réduit (22 lx) furent soumis à des essais en vue de déterminer la différence minimale d'intensité lumineuse qu'elles peuvent détecter avant et 71 h après avoir reçu oralement du DDT à des concentrations de 0, 30, 50 et 80% de la DL50. Le pouvoir discriminatoire diminue en fonction linéaire de l'augmentation de la dose, alors que la performance et la rétention ne sont pas affectées.

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ONE of the effects of DDT poisoning in fish is a heightened excitability in response to stimuli, seen as a general increase in sensitivity (Hatfield and Johansen 1972) or as an augmented sensitivity to specific stimuli such as electrical shock (Anderson and Prins 1970). Anderson (1968) found an increase in the frequency of impulses in the lateral line of brook trout (*Salvelinus fontinalis*) given DDT, but Bahr and Ball (1971) found lateral line discharge in rainbow trout (*Salmo gairdneri*) was not affected by DDT. O'Brien (1967) suggested that DDT was lethal due to its effects on sensory nerves. Because of its possible effects on the sensory system and its well known action on the central nervous system (e.g. O'Brien 1967), DDT may affect discriminating ability that involves a fine integration of both peripheral and central nervous functions. The purpose of this study was to examine the effect of DDT on visual discriminating ability in rainbow trout using operant conditioning. The experimental design also allowed examination of the effect of DDT on retention and performance of the conditioned response.

Materials and Methods

Fingerling rainbow trout obtained from the Provincial Trout Rearing Station, Raven, Alberta, were held at 15 ± 1 C on a 12L:12D photoperiod for at least 2 wk in fiberglass tanks that received a continuous flow of aerated, filtered, dechlorinated Edmonton tapwater.

The fish were trained to discriminate between a bright (723 lx) and a dim (22 lx) incandescent light using the training procedures and apparatus described by McNicholl (1974). The training apparatus was a large, T-shaped plexiglass box, fitted with stainless steel plates on the bottom, and 64 mm (2.5 inches) above them, stainless steel grids, through which a 2.1 mamp/cm^2, 60 cps AC shock could be delivered. The fish were trained six at a time to go to the right arm of the T when a bright light was presented and to the left arm when a dim light was presented. They were then each marked with a numbered fry tag placed in the musculature just anterior to the caudal fin and individually tested to make certain that they could perform the task. They were then weighed and assigned to groups that were to receive particular doses of DDT such that the groups of fish tested at each dose had similar mean weights (Table 1).

Each fish was then discrimination tested. The first stimulus presented was the bright alternated with the dim light to which the fish had been trained with a total of 10 light presentations. The order of presentation was random with from one to three presentations of the bright light alternating with from one to three of the dim light, five of each intensity given in the

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[1] Send reprint requests to W. C. Mackay.

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TABLE 1. Retention and performance scores for rainbow trout (*Salmo gairdneri*) at each of the four doses. N = 13 for all doses.

			Retention	Performance	
Dose (Toxic units)	Mean weight (g)	Weight range	Mean retention score ± 1 SD	% error before DDT	% error after DDT
Control	21.1	16.3–26.5	1.92±0.8	14.6	9.5
0.3	20.7	17.1–23.0	−1.31±1.1	11.7	13.7
0.5	21.3	17.2–25.9	−0.85±0.8	11.9	7.5
0.8	21.4	17.5–27.3	1.62±0.8	15.1	7.7

10 presentations. The light intensities were gradually brought closer together presenting the light intensities randomly and alternately, until the fish was no longer able to discriminate between the two and made 50% incorrect responses as in Hester (1968). The level of discriminating ability was the difference between the two light intensities (in lux) at which the fish made 50% incorrect responses.

The fish were then anesthetized with M.S. 222 and force fed a DDT-treated food pellet as described in McNicholl and Mackay (1975). The doses used were 0, 0.3, 0.5, and 0.8 toxic units (tu). One toxic unit is the dose required to kill 50% of the fish (see Sprague 1970), based on the incipient LD50 level of 0.03 mg DDT/g body weight found in McNicholl and Mackay (1975). Discrimination testing was again performed 71 h after DDT administration. The "change in discriminating ability" was calculated by subtracting the discriminating ability (in lux) after DDT administration from discriminating ability before DDT administration. Therefore, a negative score denotes detriment and a positive score improvement in discriminating ability.

The sequence of the first 20 light presentations was kept constant before and after DDT administration so that retention could be determined. Retention was defined as the number of errors in the first 20 trials before DDT administration minus the number of errors in the first 20 trials after DDT administration. A measure of performance was calculated by comparing the total percent error, at all light intensities above the level of discriminating ability, of all fish at a given dose level during discrimination testing before and after DDT administration.

Fish were trained and tested from November 1973 to April 1974. Times of training and testing were systematically randomized to negate any effect of time of day (Hatfield 1970) or season on conditioning or discriminating ability. It was necessary to treat most fish for fin rot; however, all fish remained healthy following treatment.

Results

As the dosage of DDT was increased, discriminating ability decreased in a linear fashion (Fig. 1). In general, the discriminating ability of control fish improved. All fish at the 0.8 tu level showed a decrease in discriminating ability, and the fish in the two intermediate groups were variable. The change in discriminating ability was analyzed for the effect of time by analysis of variance. A Duncan's Multiple Range Test revealed, in the 0.5 tu group, a significant difference ($P < 0.01$) between two of the four time periods in which testing was done. Because sample size in each time period was small (three or four), time of testing had no effect at any of the other doses, and only two of the four testing periods in the 0.5 tu group were significantly different ($P < 0.01$), it was decided to accept this as chance variation and pool the data for all testing times at each dose. A Duncan's Multiple Range Test showed both the controls and the 0.8 tu group to be significantly different from all others, and the

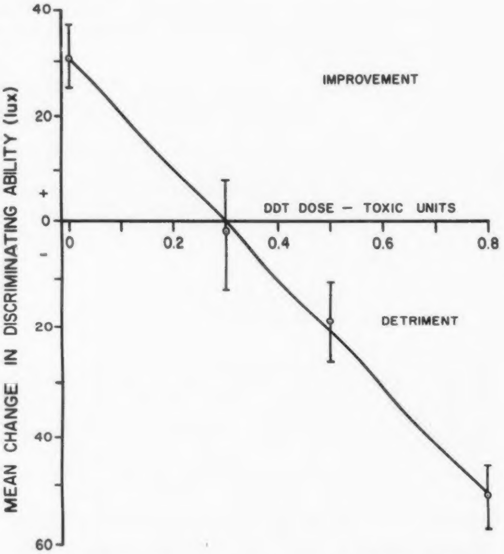

FIG. 1. Mean change ± 1 SE in discriminating ability of rainbow trout (*Salmo gairdneri*) before and after DDT administration at 0, 0.3, 0.5, and 0.8 tu.

0.3 and 0.5 tu groups to be significantly different from the others but not from each other ($P < 0.01$).

No effects of DDT were seen on retention ($P > 0.01$, Duncan's Multiple Range Test, Table 1). Performance increased (i.e. percent errors decreased) in all but one group (0.3 tu) tested following DDT administration but these increases were not significant (χ^2 range 0.35–3.62, Table 1).

Discussion

The present study indicates that DDT has the effect of decreasing discriminating ability in fish, as found in birds (Davis 1965). Thompson and Lilja (1964) found a transitory increase in auditory acuity in rats given DDT, quickly followed by a decrease. Hansen (1972) has suggested that changes in salinity selection as a result of DDT treatment in mosquitofish (*Gambusia affinis*) may have reflected changes in the fish's ability to discriminate between different salinities. That DDT can specifically decrease visual sensitivity is indicated by the finding of Alderdice and Worthington (1959) that development of blindness in coho salmon (*Oncorhynchus kisutch*) was associated with high concentration of aerial DDT spray to the test fish. The linear dose response curve found in the present study was not seen in either Davis's (1965) or Thompson and Lilja's (1964) study. Davis used three doses, the highest of which caused all the animals to die, and the other two ($\frac{1}{5}$ and $\frac{1}{25}$ of the lethal dose) both decreased discriminating ability. Thompson and Lilja (1964) used daily injected doses of DDT so the biphasic curve shown by them is not really comparable to the linear curve shown in this study with a single oral dose. Neither of these studies related the dose given to the LD50 although animals died in both.

No effect of DDT on retention of a conditioned response was evident in the present study (Table 1) as was also found by Jackson et al. (1970) in Atlantic salmon parr (*Salmo salar*) and brook trout (*Salvelinus fontinales*) and by Hatfield and Johansen (1972) in Atlantic salmon parr. In both of these studies fish were tested 24 h after DDT administration, which in itself took 24 h. Thus 48 h elapsed from the first to the second training session. DDT has been shown to affect short-term memory (Davey and Kleerekoper 1970; Davey et al. 1972). Short-term memory in goldfish appears to last between 5 and 10 s (Westlake and Kleerekoper 1970), while we define retention as long-term memory. The interval of 71 h between testing used in the present study clearly exceeds the normal range of short-term memory. However, it is difficult to determine exactly where long-term memory begins and short-term memory ends.

This study is the only one in which the effect of DDT on performance in fish has been studied; it was shown to have no effect. Variable effects have been found in mammals (for review, see Craig 1972).

We found that discrimination was adversely affected by DDT, while performance and retention were not. This may be due to the increased importance of sensory input in discriminating ability as compared to retention and performance. According to O'Brien (1967) DDT may be lethal due to its effects on sensory nerves (at least in insects). Thus the difference in effect may be due to the difference in importance of the sensory system in these functions. Central nervous functions that we can measure are virtually all tied to sensory systems, so separation of central and peripheral effects is difficult, although the studies of Davey (e.g. Davey et al. 1972) indicate that effects on the brain itself do occur.

Although the present study was a fairly specific one, involving the effect of one chemical on three specific functions, it may have wider application. First, DDT was chosen as a representative of a class of compounds, the chlorinated hydrocarbons, that are receiving widespread use as pesticides. Further research is needed to determine if the effects obtained are specific to DDT. Secondly, the behavioral response tested is only one of the myriad responses an organism can show. These behavioral responses have evolved through selection and are likely the most efficient for the survival of the organism. Any changes in behavior could result for example in decreased growth, reproduction, or survival.

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Swimming Performance of Juvenile Coho Salmon (*Oncorhynchus kisutch*) Exposed to Bleached Kraft Pulpmill Effluent

T. E. HOWARD

Division of Applied Biology, B.C. Research, Vancouver, B.C. V6S 2L2

HOWARD, T. E. 1975. Swimming performance of juvenile coho salmon (*Oncorhynchus kisutch*) exposed to bleached kraft pulpmill effluent. J. Fish. Res. Board Can. 32: 789–793.

Maximum critical swimming speeds were not achieved by fingerling coho salmon (*Oncorhynchus kisutch*) in concentrations of bleached kraft pulpmill effluent above a threshold concentration between 10–20% of the 96-h LC50. Reduction of swimming performance was related to effluent concentration but not to exposure time after the initial 18 h. Swimming performance returned to control levels for fish tested in water after 6–12 h recovery from exposure to effluent concentrations up to 0.7 LC50. Effluent was rendered nontoxic by aerobic microbiological fermentation but some reduction in swimming performance was measured which was attributed, in part, to the effects of color and lignin materials remaining after treatment.

The results are discussed in relation to the mode of toxic action of bleached kraft pulpmill effluents and their possible impact on aquatic ecosystems.

HOWARD, T. E. 1975. Swimming performance of juvenile coho salmon (*Oncorhynchus kisutch*) exposed to bleached kraft pulpmill effluent. J. Fish. Res. Board Can. 32: 789–793.

Les alevins de la grosseur du doigt de saumon coho (*Oncorhynchus kisutch*) n'atteignent pas la vitesse de nage critique maximale lorsque placés dans des concentrations d'effluent de blanchiment de moulin de pâte à papier kraft dépassant un seuil de 10–20% de la CL50 après 96 h. La diminution de performance natatoire est liée à la concentration de l'effluent, mais non à la durée de l'exposition après une période initiale de 18 h. La performance natatoire se rétablit au niveau de celle des témoins chez des poissons soumis à des essais dans l'eau après 6–12 h de récupération d'une exposition à des concentrations d'effluents allant jusqu'à 0.7 de la CL50. L'effluent devient non toxique à la suite d'une fermentation microbienne aérobie, mais on observe une certaine diminution de la performance natatoire attribuable en partie aux effets de couleur et de matériaux ligneux encore présents après traitement.

Nous analysons ces résultats en relation avec le mode d'action toxique des effluents de blanchiment de moulin de pâte à papier kraft et leurs effets possibles sur les écosystèmes aquatiques.

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THIS report documents effects of bleached kraft pulpmill effluent (BKME) on the sustainable swimming performance of juvenile coho salmon (*Oncorhynchus kisutch*). Critical swimming speeds (Brett 1964, 1967) were measured in sublethal concentrations of BKME between 10 and 90% of the 96-h median lethal concentration (LC50) after exposure to effluent for 7 to 168 h. Critical swimming speeds were also measured in water after exposure to effluent and in BKME that had been detoxified by aerobic microbiological fermentation in treatment systems similar to those in use at a number of bleached kraft mills.

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Materials and Methods

Effluent was collected from three B.C. mills that were producing fully bleached kraft pulp by conventional processes (mills C, F, and G; Howard and Walden 1971). To prevent loss of effluent toxicity the samples were stored at 2 C in completely filled containers; immediately before use the samples were filtered through cloth to remove fiber, and the pH adjusted to 7.1–7.5. The 96-h LC50 of each sample of BKME was measured by conventional bioassay (American Public Health Association 1971) in 40-liter containers with minimal aeration to maintain the dissolved oxygen content at saturation and with a maximum of 0.7 g wet weight of fish per liter of test solution. The concentration of BKME used in the swimming studies was expressed as a proportion of the 96-h LC50. The range of LC50 values for BKME was between 18.5 and 45% vol/vol.

TABLE 1. Mean critical swimming speeds (L/s ± 1 SD) for fingerling coho salmon (*Oncorhynchus kisutch*) in sublethal concentrations of BKME after exposure to the effluent for 0–168 h. (10 fish per group; mean length of fish, 6.43 cm.)

Effluent exposure (*h*)	Concentration of BKME as proportion of 96-h LC50					
	0 (control)	0.1 LC50	0.2 LC50	0.4 LC50	0.5 LC50	0.9 LC50
0	5.63	6.03	5.43	5.22	4.21[a]	2.60[a]
	(0.54)	(0.54)	(1.18)	(0.88)	(0.44)	(0.50)
8	6.54	5.74	5.00	5.05	4.66[a]	2.40[a]
	(0.58)	(0.93)	(1.01)	(1.42)	(0.83)	(0.49)
18	6.53	6.24	4.70[a]	3.90[a]	3.94[a]	1.83[a]
	(0.40)	(0.36)	(0.85)	(0.90)	(1.52)	(0.65)
48	5.55	5.23	4.68[a]	3.59[a]	2.20[a]	1.28[a]
	(0.52)	(0.75)	(0.40)	(0.36)	(0.51)	(1.05)
96	5.73	5.74	4.73[a]	3.56[a]	2.28[a]	1.73[a]
	(0.39)	(0.53)	(0.33)	(0.32)	(0.42)	(0.82)
168	5.48	5.42	4.34[a]	3.50[a]	2.06[a]	1.18[a]
	(0.54)	(0.44)	(0.41)	(0.21)	(0.34)	(0.65)

[a] Significant difference in ordered means at $P < 0.05$ by Tukey's test.

Nontoxic BKME was obtained in the laboratory by fermentation of the original waste in a Benedict-type activated sludge unit operated on 8- or 24-h detention time at 20 ± 1 C and pH 7.1–7.5. Nutrients were added in the ratio 100 (BOD_5 initial):5 (N):1 (P). Detoxified effluent was also produced from pilot-scale aerated stabilization lagoons with 1-, 3-, or 5-day detention time, operated at Northwood Pulp and Paper, Prince George, B.C. (Mueller and Walden 1974).

The fish used were 90–240 day fingerling coho obtained as swim-up fry from the Samish Hatchery in Washington State. Mean fork length of the stock population increased from 3.4 to 9.1 cm during the testing program. The fish were fed Oregon moist pellets, maintained at low population density at 11–13 C with a natural daily photoperiod, and trained against a current of 7–13 cm/s. Dechlorinated Vancouver city water was used in fish maintenance and for the preparation of test solutions (pH 6.5–6.8, conductance 8–24 micromho/cm, EDTA hardness 4–9 mg $CaCO_3$/liter, iron <0.2 mg/liter, heavy metals <5 μg/liter).

During the test program, critical swimming speed of the stock fish was measured regularly and the standard deviation was less than ±10% of the mean value. No fish was tested more than once.

The coho were preexposed to BKME in 40-liter containers and transferred to the stamina testing system 1–3 h before a test of swimming ability. During effluent exposure the solutions were changed at 24-h intervals and the fish were fed just before the solution exchange. Food was withheld for 24 h preceding the measurement of critical swimming speed. There was no indication of depressed appetite even at high concentrations of BKME.

The apparatus (Howard 1973) functioned similarly to systems used by other workers for the measurement of sustained performance (Brett 1964; Thomas et al. 1964). Solution was recycled at 13 ± 0.5 C from a 300-liter reservoir through three tunnels (9 cm ID, 95 cm long). In each tunnel, an expansion cone and straightening vanes provided nonrotating microturbulent flow at test speeds.

Critical swimming speed was measured in lengths per second (L/s) following the procedures developed by Brett (1964). Initial velocity was 0.8 L/s and the speed was increased, in steps of approximately 1.6 $L^{0.6}$, at 60-min intervals to the fatigue velocity. A minimum of 10 fish was used to obtain the mean and standard deviation of critical swimming speed for each effluent concentration and exposure time.

Analysis of variance was used to examine the effects of effluent concentration and exposure time on critical swimming speed. Tukey's test was chosen as a conservative measure of significance between the means of pairs of observations (Dunnett 1955).

Results

EFFECTS OF CONCENTRATION AND EXPOSURE TIME

Critical swimming speeds were reduced ($P <$ 0.05, Tukey's test) at BKME concentrations of 0.2 LC50 or greater after 18-h exposure (Table 1). Effluent concentration was the main factor affecting swimming performance, and extending the exposure period from 18 to 168 h caused no further reduction for any single concentration of BKME. At short exposure times the differences due to concentration were less clear-cut. Performance was unaffected by exposure to a BKME concentration of 0.1, LC50.

RECOVERY FROM EFFLUENT

The effects of BKME on swimming performance were fugative and fish recovered quite

TABLE 2. Critical swimming speeds (L/s ± 1 SD) for fingerling coho salmon with no recovery period after exposure to BKME for 1–110 h. (15 fish per group; mean length of fish, 8.41 ± 0.43 cm.)

Effluent exposure (*h*)	Concentration of BKME as proportion of 96-h LC50		
	0 (control)	0.36 LC50	0.72 LC50
1	4.92±0.41	4.09±0.40[a]	4.16±0.34[a]
8	4.83±0.31	3.62±0.29[a]	3.64±0.59[a]
18	5.16±0.47	3.62±0.49[a]	3.51±0.42[a]
110	4.90±0.37	2.95±0.21[a]	2.85±0.84[a]

[a]Significant reduction at $P < 0.05$ by one-tailed Student's "*t*" comparison with corresponding control.

TABLE 3. Critical swimming speeds (L/s ± 1 SD) for fingerling coho salmon after recovery for 0–24 h following exposure to BKME for 18 h. (15 fish per group; mean length of fish. 8.31 ± 0.42 cm.)

Recovery time (*h*)	Concentration of BKME as proportion of 96-h LC50			
	0 (control)	0.15 LC50	0.35 LC50	0.70 LC50
0	4.58±0.37	4.37±0.42	3.30±0.40[a]	2.82±0.38[a]
6	4.53±0.12	4.57±0.31	4.31±0.19	3.71±0.35[a]
12	4.32±0.40	4.53±0.26	4.53±0.30	4.31±0.50
24	4.57±0.25	4.57±0.46	4.25±0.36	4.03±0.37

[a]Significant reduction at $P < 0.05$ by one-tailed Student's "*t*" comparison with corresponding control group.

rapidly when they were transferred to clean water.

Critical swimming speeds in water were depressed for fish tested in the stamina system directly after exposure to 0.36 or 0.72 LC50 concentrations of BKME for 1–110 h (Table 2). This reduction of swimming performance was apparently independent of effluent concentration. However, the concentration of BKME to which the fish were exposed did influence the recovery time needed before swimming speeds returned to control levels (Table 3). At a relatively low BKME concentration (0.35 LC50), 6 h were needed, whereas 12-h recovery was required after exposure to a BKME concentration of 0.70 LC50.

PERFORMANCE IN DETOXIFIED EFFLUENT

Aerobic fermentation can remove the dissolved organic toxicants in BKME as shown in Table 4. After detoxification by activated sludge treatment, critical swimming speeds (Table 5) were reduced only when the concentration was 100% vol/vol, equivalent to 2.5 LC50 of the original effluent sample. The laboratory fermenter used provides the best available treatment for BKME (Mueller and Walden 1974) and reduction in swimming performance is attributed to the effects of color and lignin materials that are virtually unaffected by aerobic fermentation. Pilot-scale aerated stabilization was less effective in this case; swimming performance was reduced at a BKME concentration equivalent to 1.0 LC50 of the original effluent. Here, the reduction may be due in part to organic toxicants remaining after treatment but at a concentration below that causing acute toxicity to the fish.

Discussion

The threshold concentration of BKME that prevented coho from achieving maximum critical swimming speeds was similar to levels that affect cough frequency and respiration (Walden et al. 1970; Davis 1973), growth (Webb and Brett 1972), temperature tolerance (Howard 1973), and biochemistry (McLeay 1973). Quantitative impairment of swimming performance and several of the effects listed above are the type of ecologically significant response that Sprague (1971) pointed out was necessary to measure

TABLE 4. Characteristics of BKME samples before and after detoxification by activated sludge treatment or aerobic lagoon.

			Detoxified	
		Untreated	Activated sludge	Aerobic lagoon
Sample 1	pH	6	7.1	7.1
	conductance (micromho/cm)	3750	3150	3600
	sodium (mg/liter)	620	600	525
	BOD_5 (mg/liter)	307	81	40
	color (APHA units)	3600	3600	3600
	resin acids (mg/liter)[a]	4.16	0.29	0.81
	fatty acids (mg/liter)[a]	0.71	0.58	0.63
	median survival time at 100% vol/vol (min)	100	nontoxic	nontoxic
	96-h LC50 (% vol/vol)	40	nontoxic	nontoxic
Sample 2	pH	7.0	7.0	7.0
	conductance (micromho/cm)	2920	2750	2400
	sodium (mg/liter)	400	362	344
	BOD_5 (mg/liter)	228	97	37
	color (APHA units)	2900	2800	2800
	resin acids (mg/liter)[a]	3.08	0.33	0.91
	fatty acids (mg/liter)[a]	0.43	0.44	0.64
	median survival time at 100% vol/vol (min)	145	nontoxic	nontoxic
	96-h LC50 (% vol/vol)	65	nontoxic	nontoxic

[a] Analyzed by method of Leach and Thakore (1973).

TABLE 5. Critical swimming speeds (L/s ± 1 SD) for fingerling coho salmon in BKME and in detoxified BKME produced by aerobic microbiological fermentation. (15 fish per group; mean length of fish 8.42 ± 0.42 cm; exposure time 18 h.)

Concentration (proportion of 96-h LC50 original effluent)			Detoxified BKME		
	Untreated BKME		Activated sludge		Aerobic lagoon
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 2
0 (control)	4.13 (0.40)	4.32 (0.31)	—	—	—
0.2 LC50	3.87 (0.41)	4.16 (0.45)	—	—	—
0.5 LC50	2.26* (0.78)	2.32* (0.49)	—	—	—
0.7 LC50	—	—	—	—	4.00 (0.54)
1.0 LC50	1.20[a] (0.02)	1.91[a] (1.10)	4.30 (0.68)	—	3.26[a] (0.71)
1.5 LC50	—	—	—	3.91 (0.62)	3.08[a] (0.48)
2.5 LC50	—	—	—	—	2.81[a] (0.24)

[a] Significant reduction at $P < 0.05$ by one-tailed Student's "*t*" with the control group.

before safe limits can be set for pollutants entering aquatic ecosystems. With general agreement concerning the threshold concentration causing sublethal responses, we should now be concerned with the mode of action and environmental effect of BKME.

Most workers have stated that BKME causes respiratory stress (e.g. Alderdice and Brett 1957; Walden et al. 1970), while Davis (1973) showed that BKME exposure induced "... ventilatory abnormality and reduced saturation of the arterial blood." My findings, under conditions of maximum exercise, indicate that BKME impairs the ability of fish to transfer oxygen across the gills.

Resin and fatty acids are important toxicants in BKME (Leach and Thakore 1973); the fugative nature of the effect on swimming performance supports the view that the dissolved organic toxicants in BKME may be adsorbed or bound by a weak chemical bond to the gill epithelium and after transfer to fresh water, desorption may occur or the toxicants may be metabolized by the fish. In unpublished studies, Thakore and Farvolden (1973) showed that injections of fatty and resin acids into the dorsal musculature did not kill fish within 96 h and that if fish were exposed to radioactively labelled fatty acids, the activity was concentrated in the phospholipid fraction of gill extract.

The data presented here relate to the possible effects of BKME or other pollutants upon salmonids, particularly upstream migrants. The locations of industrial outfalls should be selected to avoid high concentrations of effluent in areas which are preferred migration routes of salmon passing the point of discharge. Upstream migration involves a rate of travel above that at which energy is most efficiently utilized for locomotion; moreover, available energy reserves of the fish and the time required for upstream migration are in delicate equilibrium for the populations of each spawning area. A typical bleached kraft pulpmill will discharge effluent at a rate of approximately 20 cfs. Assuming that without treatment this effluent has a 96-h LC50 of 25% vol/vol and the threshold affecting swimming performance is 0.15 LC50, then dilution with 534 cfs of river flow is required to prevent obvious hazard to fish migration. Installation of an effective waste treatment system would substantially increase the safety factor.

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Mercury in Seals, Terrestrial Carnivores, and Principal Food Items of the Inuit, from Holman, N.W.T.

THOMAS G. SMITH

Department of the Environment, Fisheries and Marine Service, Arctic Biological Station, Ste. Anne de Bellevue, Que. H9X 3L6

AND F. A. J. ARMSTRONG

Department of the Environment, Fisheries and Marine Service, Freshwater Institute, Winnipeg, Man. R3T 2N6

SMITH, T. G., AND F. A. J. ARMSTRONG. 1975. Mercury in seals, terrestrial carnivores, and principal food items of the Inuit, from Holman, N.W.T. J. Fish. Res. Board Can. 32: 795–801.

Total mercury analyses were made on liver and muscle of ringed and bearded seals, of caribou, and on muscle of Arctic char (the principal items in the diet of local people), of Arctic fox and sledge dogs (which feed mostly on ringed seal) and on wolves (predacious on caribou). About half of the 123 samples were also analyzed for methyl mercury, and some for selenium.

Ringed seals showed mean total mercury levels of 27 ppm in liver and 0.73 in muscle. Bearded seals showed 143 in liver and 0.53 in muscle. In the livers, methyl mercury was a small fraction of the total, being 5.6% in ringed and 0.38% in bearded seals. There were positive and significant correlations of mercury content with age and body weight. In Arctic char, mean total mercury was 0.049 ppm, and in caribou 0.20 in liver and 0.017 in muscle. In Arctic fox, values for liver and muscle were 0.77 and 0.32, and in sledge dogs 11.5 and 0.79 ppm.

Seals form only part of the diet of the local people. Blood mercury levels have been reported for this community as being above average though not dangerously so. A change in diet, such as increased consumption of seals, might change this.

SMITH, T. G., AND F. A. J. ARMSTRONG. 1975. Mercury in seals, terrestrial carnivores, and principal food items of the Inuit, from Holman, N.W.T. J. Fish. Res. Board Can. 32: 795–801.

Nous avons fait des analyses de mercure total sur le foie et le muscle de phoques annelé et barbu, de caribou, sur le muscle d'omble chevalier (items principaux du régime alimentaire des autochtones), du renard arctique et des chiens de traîneau (qui se nourrissent surtout de phoque annelé) et sur le loup (prédateur du caribou). Nous avons de plus déterminé le mercure-méthyl sur environ la moitié des 123 échantillons et le sélénium sur quelques-uns.

Le phoque annelé montre des niveaux moyens de mercure total de 27 ppm dans le foie et 0.73 dans le muscle. Le phoque barbu, 143 dans le foie et 0.53 dans le muscle. Dans les foies, le mercure-méthyl représente une faible proportion du total, soit 5.6% chez le phoque annulé et 0.38% chez le phoque barbu. Il y a une corrélation positive et significative entre le contenu en mercure et l'âge et le poids corporel. Chez l'omble chevalier, le mercure total moyen est de 0.049 ppm et chez le caribou, 0.20 dans le foie et 0.017 dans le muscle. Chez le renard arctique, les valeurs sont de 0.77 dans le foie et 0.32 dans le muscle, alors qu'elles sont de 11.5 et 0.79 ppm pour les chiens de traîneau.

Les phoques ne constituent qu'une faible partie du régime des autochtones. On a signalé dans cette communauté des niveaux de mercure sanguin au-dessus de la moyenne, mais non au point dangereux. Un changement de régime, telle une consommation accrue de phoques, pourrait modifier cette situation.

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EARLY studies of mercury content in the tissues of organisms used as human food assumed that high mercury levels were directly attributable to man-induced pollution. More recently, analyses of mercury content from animals living at great distances away from industrial activities, and completely isolated from them, have shown that mercury is often accumulated from the uncontaminated environment.

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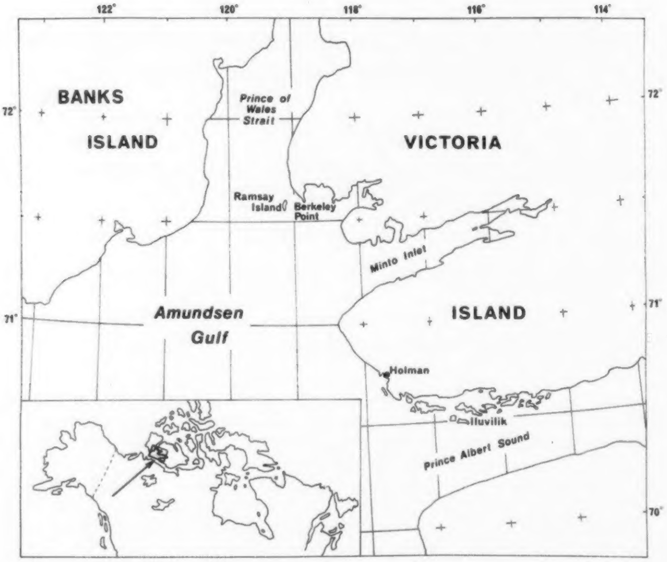

FIG. 1. Location of Victoria Island, N.W.T.

Marine mammals have been shown to contain high mercury levels in certain tissues (Helminen et al. 1968; Heppleston and French 1972; Gaskin et al. 1972; Sergeant and Armstrong 1973; Anas 1974). Henriksson et al. (1969) reported very high total mercury levels in the liver of a ringed seal *Phoca hispida saimensis* from a freshwater lake in Finland.

In spring 1972, a sample of tissues from the ringed seal *Phoca hispida hispida*, was collected from Prince Albert Sound, near the village of Holman on western Victoria Island, N.W.T. High total mercury levels in the livers caused one of us (T.G.S.) to expand the study in 1973 to include bearded seals (*Erignathus barbatus*), caribou (*Rangifer tarandus*), and Arctic char (*Salvelinus alpinus*), which comprise the principal items in the local native diet. The restricted diet of the Inuit, especially when considered on a seasonal basis, made it important to assess the presence and content of mercury in their food, particularly since a recent report (Anon. 1972) has shown that blood mercury levels in this community are higher than average, though by no means dangerously so.

Samples were also obtained of tissues from the Arctic fox (*Alopex lagopus*) and Inuit sledge dogs (*Canis familiaris*) which feed almost exclusively on ringed seals. It was also possible to collect tissues from a number of Arctic wolves (*Canis lupus*) which are primarily terrestrial predators feeding mainly on caribou.

Material and Methods

All species sampled were obtained from the area regularly hunted by the natives of Holman. This includes the coastline as far north as Berkeley Point and as far south as Iluvilik (Fig. 1). Specimens were obtained from seals that were shot or from foxes that were trapped by the Inuit during the spring months when they were feeding to a large extent on ringed seal pups.

Liver and muscle were sampled since both are eaten as regular food items by the Inuit and because liver has been shown to be high in mercury in seals (Sergeant and Armstrong 1973; Anas 1974) and other mammalian species. Samples were frozen and packaged in polyethylene bags. These were kept frozen until analyzed at the Freshwater Institute, Winnipeg. Portions of the tissues were analyzed by the method of Armstrong and Uthe (1971) for total mercury, by that of Uthe et al. (1972) for methyl mercury, and by that of Hoffman et al. (1968) for selenium. Results are expressed as ppm (Micrograms per gram Hg) on a wet weight basis.

Age determination of ringed seals follows the methods described by McLaren (1958a) and Smith (1973). Bearded seals were aged using the claws from the foreflippers as in McLaren (1958a).

Results

Average total mercury and methyl mercury contents in liver and muscle tissues for all species considered are shown in Table 1. Large standard deviations are explained by strong positive correlations of mercury content with age and in some cases with weight of the animal.

RINGED SEAL

The highest total mercury value of 184.0 ppm was obtained from the liver of a 17-yr-old female animal shot near Iluvilik in spring 1973. A 30-yr-old male showed the highest total mercury value of 2.12 ppm in the muscle. The highest value for methyl mercury in the liver was obtained from a 25-yr-old male seal. This represents 18% of the total mercury in the liver. The average percentage of methyl mercury in the liver tissues of 40 specimens was 5.61. Only six samples of muscle were analyzed for methyl mercury and the average percentage was 72.34 (SD = 15.22).

Regressions of total mercury on age show significant correlations for the 1972 female samples of liver tissues ($r = 0.72$, $P < 0.01$, $F = 18.77$, $P < 0.001$), and for the combined 1972 and 1973 male and female muscle samples ($r = 0.39$, $P < 0.01$, $F = 14.46$, $P < 0.001$). Although the 1972 male liver samples do not show a significant reduction of variation of total mercury on age, this same sample is significant when a regression of total mercury on body weight is run ($r = 0.46$), $P < 0.05$; $F = 6.11$, $P < 0.025$). In the livers of the 1972 females, the regression of total mercury on weight is also significant ($r = 0.50$, $P < 0.05$; $F = 5.945$, $P < 0.05$). The 1973 tissue samples from liver and muscle do not show significant regressions on either age or body weight. This is explained by the absence in this sample of animals less than 4 yr of age and the high variability of total mercury content in both liver and muscle of the adult specimens.

In 1973, methyl mercury analyses were carried out on the liver tissue. The combined male and female samples show a significant regression of methyl mercury on age ($r = 0.38$, $P < 0.05$; $F = 6.68$, $P < 0.025$). No significance was found in the same sample for methyl mercury on body weight.

Mean selenium values of 15.52 ppm (SD = 7.82) and 1.06 ppm (SD = 0.35) were found in the liver and muscle of ringed seals. Koeman et al. (1973) show a 1:1 molar ratio of selenium and mercury for the livers of small cetaceans and harbour seals collected in several different localities. The present study shows the same relationships with regressions of selenium (ppm) on mercury (ppm) of $y = -22.93 + 4.09x$ ($r = 0.924$, $P < 0.01$ and $F = 238.18$, $P < 0.0001$) and mercury on selenium of $y = 6.05 + 0.23x$ ($r = 0.917$, $P < 0.01$ and $F = 206.84$, $P < 0.001$).

BEARDED SEAL

The highest mercury level recorded during this study, 420.00 ppm, was from the liver of a 9-year-old female bearded seal. This same specimen contained 88.7 ppm total mercury in the muscle which was also the highest level for this species. Methyl mercury in the liver was very low averaging 0.38% for the six specimens. No significant correlations between total mercury or methyl mercury and age were observed because of the small sample size. A mean level of 34.0 ppm (SD = 33.02) of selenium was found in the liver.

ARCTIC CHAR AND CARIBOU

Of the other two principal food items of the Holman Inuit, it was possible to obtain only a known age sample of Arctic char. The highest total mercury level was obtained from the muscle of a 23-yr-old char which was the oldest specimen in the sample. Regressions of total mercury on age and body weights for char were both significant ($r = 0.819$, $P < 0.01$; $F = 20.36$, $P < 0.0001$ and $r = 0.901$, $P < 0.01$; $F = 43.32$, $P < 0.001$, respectively).

Only three samples of caribou were obtained; all were adult animals and two were pregnant females. Total mercury levels in both liver and muscle were very low, the highest levels being in the liver (Table 1).

FOX, SLEDGE DOG, AND WOLF

Of 16 Arctic foxes sampled, the highest mercury level of 4.25 ppm came from the liver of an adult male specimen. Average values (Table 1) were much lower with the highest levels being found in the liver tissues.

Twelve samples of tissues were collected from Inuit sledge dogs, which are fed an almost exclusive diet of ringed seals. These displayed the highest mercury levels of the terrestrial carnivores with a maximum total mercury load of 30.3 ppm in the liver and 1.06 ppm in the muscle. Seven Arctic wolves, which are primarily terrestrial carnivores feeding on caribou and small mammals, showed a maximum value of only 0.68 ppm in the liver and 0.08 in the muscle tissue.

Discussion

Maximum and mean values of total mercury levels from other species of pinnipeds and cetaceans in various other studies are shown in Table 2. Of the species taken from the Canadian arctic which are presumed to be far removed from industrial contamination, the ringed seals from the present study show the highest maximum and average total mercury concentrations. Harp seals, which spend half their lives in arctic regions and the other half in the Gulf of the St. Lawrence, thus

TABLE 1. Total mercury and methyl mercury (ppm) in seals, Arctic char, caribou, Arctic fox, sledge dogs, and wolves.

	Total mercury in liver			Methyl mercury in liver			Total mercury in muscle			Methyl mercury in muscle		
Species	N	$\overline{X}$	SD	N	$\overline{X}$	SD	N	$\overline{X}$	SD	N	$\overline{X}$	SD
Phoca hispida (ringed seal)	80	27.5	30.1	40	0.96	0.45	80	0.72	0.33	6	0.83	0.31
Erignathus barbatus (bearded seal)	6	143	170	6	0.30	0.25	3	0.53	0.35			
Salvelinus alpinus (Arctic char)							12	0.049	0.017			
Rangifer tarandus (caribou)	3	0.20	0.036				3	0.017	0.006			
Alopex lagopus (Arctic fox)	16	0.76	1.12				16	0.31	0.54			
Canis familiaris (sledge dog)	11	11.4	8.8	11	0.86	0.25	11	0.79	0.16			
Canis lupus (wolf)	7	0.24	0.15				7	0.051	0.027			

presumably being exposed to some level of industrial mercury contamination, also show mercury levels lower than the specimens in this study. Ringed seals (*Phoca hispida botnica*) from the Gulf of Finland, which are presumably subject to contamination from chlor-alkali factories, also show lower concentrations. Both ringed and bearded seals from the Bering Sea and Chukchi Sea have much lower concentrations (W. Galster personal communication) than those seen in this study. Only ringed seals from Lake Saimaa, a relatively small closed system surrounded by industrial sources of mercury, yields higher mercury concentrations. Samples of ringed seal tissues collected in other Canadian arctic localities show much lower values, suggesting a local concentration of mercury bearing sediments as a possible explanation for the high levels seen in this study. This is also suggested by the even higher concentrations of mercury in the bearded seals taken during this investigation. This species is generally believed to be sedentary. Its distribution is restricted by its dependence on concentrations of benthic invertebrates on which it feeds. The high content of mercury in the sediments in these feeding areas could explain the values found for this species. It is also possible that there is more mercury accumulated by the longer lived benthic invertebrates used as food by the bearded seal. Recently Gaskin et al. (1974) also suggested concentrations of mercury-bearing ores as a possible explanation for mercury levels in pilot whales *Globicephala macrorhynca* and long-snouted dolphins *Stenella longirostris* (?) from waters near St. Lucia, Lesser Antilles.

This study and several others (e.g. Heppleston and French 1972; Sergeant and Armstrong 1973; Anas 1974) show that mercury content in liver and muscle tissues increases significantly with the age of seals. It is unclear whether accumulation of mercury through the food chain is an important mechanism, as assumed by Sergeant and Armstrong (1973). Williams and Weiss (1973), from a detailed study of amounts of mercury in a trophic pyramid, concluded that mercury is accumulated with time in long-lived animals, and that the food chain accounts for very little magnification of these levels.

Seals exposed to high amounts of industrially produced mercury such as the harbour seals along the Netherlands coast (Koeman et al. 1973) show definite pathological signs. Maximum mercury values from the Netherlands area were much higher, often 20–50 times as great as values found in other areas (Table 2). Most studies of mercury levels in marine mammals including the present investigation show surprisingly high levels of mercury in liver tissues, but there are no obvious toxic or pathological signs evident. In the case of seals exposed to sources of industrially produced mercury, the type of mercury ingested and its synergistic, additive, or complementary relationships with other industrial contaminants are probably very important.

Ganther et al. (1972) indicated the decreased toxicity of methyl mercury in diets containing selenium. Parizek and Ostadalova (1967) and Parizek et al. (1971) showed experimentally that selenium reacting with mercury in the body changed the biological effect of both metals. They further suggested that the passage of mercury via the blood to the foetus and milk to the newborn

TABLE 2. Total and average mercury levels (ppm) in liver tissues of marine mammals from different study areas.

Study	Area	Species	Total mercury (ppm)		
			Maximum	Mean	n
Sergeant and Armstrong (1973)	Canada; St. Lawrence River Estuary	*Pagophilus groenlandicus*	10	3.63	20
	Gulf of the St. Lawrence River	*Cystophora cristata*	45	37.2	3
	Sable Island	*Phoca vitulina*	21	8.86	8
	Sable Island	*Halichoerus grypus*	387	88.2	11
Bligh and Armstrong (1971) (unpublished MS)	Canada; Hudson Bay	*Delphinapterus leucas*		0.53	43
	Eastern arctic	*Monodon monoceros*		0.64	2
Present study (1972–73)	Canada; Amundsen Gulf	*Phoca hispida hispida*	184	27.5	80
Unpublished data (1972)	Canada; Repulse Bay	*Phoca hispida hispida*	7.07	3.43	4
	Cornwallis Island	*Phoca hispida hispida*	5.72	2.93	3
Henriksson *et al.* (1969)	Finland; Lake Saimaa	*Phoca hispida saimensis*	209	55	8
	Finnish Gulf	*Phoca hispida botnica*	27.6	11	16
Anas (1974)	USA; Washington coast	*Callorhinus ursinus*	172		
	San Miguel Is., California	*Phoca vitulina*	700	269	4
Koeman *et al.* (1973)	Netherlands	*Phoca vitulina*	765		
		Phoca vitulina	935	340	16
		Halichoerus grypus	435		

was inhibited by the presence of selenium. This was borne out by the findings of Freeman and Horne (1973) where 10 harp seal pups had lower concentrations of methyl mercury than those of their mothers in all tissues but the liver. This is contrary to the finding of Berglund and Berlin (1969) for humans, where methyl mercury is readily passed through the placental barrier and concentrated in the tissues of the foetus. The known detoxifying effects of selenium (Krehl 1972) coupled with its high correlation with mercury values found in this study, indicate that this may be a factor contributing to the ability of marine mammals to tolerate high levels of this metal with no apparent toxic effects. It has been speculated that the common affinity of mercury and selenium for sulfur suggests an enzyme system that may demethylate mercury. This seems reasonable since the bulk of mercury taken in by the ringed seal comes from fish tissues that are known to contain high concentrations of the methylated form (Bache et al. 1971; Keckes and Miettinen 1973). The low concentrations of methylated mercury in the liver in this and other studies of marine mammals (e.g. Gaskin et al. 1973; Gaskin et al. 1974), further suggests this as a site for the detoxification.

The accumulation of mercury by organisms used as human food has had lethal and sublethal consequences (e.g. Berglund and Berlin 1969). All such reported cases have resulted from increased mercury levels from industrial or agricultural activities. To date, no definite report of mercury toxicity in humans exists in regions where human food species are free from man-increased levels of mercury. This is so in the present study area. However, in a study of the mercury levels in the blood of humans from 42 different communities in western and northwestern Canada (Anon. 1972), the people of Holman, the village in the present study, had the third highest mean values. Of 23 people tested, the mean blood mercury level was 34.01 ppb. There was no apparent correlation of total mercury with age in the sample combining both sexes ($y = 31.09 + 0.09x$; $r = 0.109$, $P > 0.05$). Although the Holman Inuit eat ringed seal as a regular part of their diet, they are not restricted to this food since char, caribou, lake trout, and several species of waterfowl are available in abundance either throughout the year or seasonally. It is apparent, however, that the diet of the Inuit in the Beaufort Sea area who use marine mammals as food items, contains higher mercury levels than in other areas. The Tuktoyaktuk Inuit who feed heavily on beluga meat and blubber showed the highest mean value of 37.76 ppb in the study (Anon. 1972).

The FAO/WHO Expert Committee on Food Additives, April 4–12, 1972, in Geneva, established a tolerable weekly intake of 0.3 mg total mercury, of which no more than 0.2 mg should be methyl mercury, per person per week. On this basis and using the mean values for total and methyl mercury content found in this study (Table 1) it would take only 11.1 g of liver or 312.5 g of muscle to be eaten in 1 wk for a person to exceed the provisional tolerable weekly intake. These are unrealistically small quantities; during the late spring months at Holman, when caribou has become scarce and char and waterfowl are not yet available, an exclusive ringed seal diet is the rule. The blood levels of mercury in the Inuit do not suggest that the people of this area are in danger since Swedish studies have shown that the lowest whole blood concentrations where toxic signs have appeared are approximately 200 ppb. The high natural concentrations of mercury in the seals of this area and the documented higher mercury levels in the blood of the local natives point to a potentially dangerous situation if these people were to become entirely dependent on seals for food. The low values of mercury in caribou and Arctic char (Table 1), which form their main winter and spring food, makes the overall diet appear safe. It is instructive to look at the high liver concentrations of mercury in the Inuit sledge-dogs that feed exclusively on ringed seal. This might provide some insight into what the levels would be in humans dependent on a restricted seal diet.

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Selenium Toxicity on the Early Life Stages of Zebrafish (*Brachydanio rerio*)

A. J. NIIMI AND Q. N. LAHAM

Department of Biology, University of Ottawa, Ottawa, Ont. K1N 6N5

NIIMI, A. J., AND Q. N. LAHAM. 1975. Selenium toxicity on the early life stages of zebrafish (*Brachydanio rerio*). J. Fish. Res. Board Can. 32: 803–806.

Embryos at four stages of development and the newly hatched larvae of zebrafish (*Brachydanio rerio*) were exposed to selenium concentrations between 0.5 and 10 μg/ml to establish the toxic levels at the different stages. Embryo mortality was negligible at all selenium concentrations. Following hatching, mortality among the larvae sharply increased at concentrations of 3 μg/ml or greater irrespective of the embryonic stage when exposed. For most treatments, over 90% of the larvae died within 10 days of hatching. The mortality rate for larvae exposed to selenium after hatching was slightly less.

NIIMI, A. J., AND Q. N. LAHAM. 1975. Selenium toxicity on the early life stages of zebrafish (*Brachydanio rerio*). J. Fish. Res. Board Can. 32: 803–806.

Des embryons à quatre stades de développement et les larves nouvellement écloses de poisson-zèbre (*Brachydanio rerio*) ont été exposés à des concentrations de selenium de 0.5 à 10 μg/ml en vue de déterminer les niveaux toxiques à différents stades. Les mortalités d'embryons sont négligeables à toutes les concentrations. Après éclosion, les mortalités des larves augmentent brusquement à des concentrations de 3 μg/ml ou plus, quel que soit le stade embryonnaire d'exposition. Pour la plupart des traitements, plus de 90% des larves meurent en dedans de 10 jours après éclosion. Le taux de mortalité des larves exposées au selenium après éclosion est légèrement inférieur.

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SELENIUM is a nonmetallic element with a ubiquitous distribution in nearly all materials in the earth's crust. Its concentration in the aquatic environment is associated primarily with leaching from the surrounding drainage system and the pH of the water. Summarizing the selenium content of the ground and surface waters in the United States, Larkin and Davidson (1967) reported a median value of 1 μg/liter with occasional values up to 9000 μg/liter.

Information on the acute biological effects of this element is generally limited to birds and mammals although its effects on fishes have been reported (Ellis et al. 1937; Weir and Hine 1970; Huckabee and Griffith 1974). Additional information on the biochemical effects of selenium in the aquatic environment would be highly relevant in view of the apparent protective effect from cadmium and mercury toxicity afforded by selenium (Parizek et al. 1971). This study examines the effect of selenium on several embryonic and early larval stages of zebrafish (*Brachydanio rerio*) to establish the toxic levels at different life stages.

Materials and methods — The procedures for maintaining and breeding zebrafish have been described (Niimi and LaHam 1974). Females were bred at 5–10 day intervals to ensure low embryo mortality and uniform hatching rates. Following spawning, the eggs were examined and the unfertilized and abnormally developing eggs removed. Groups of 50 embryos each were placed in 60 × 15 mm petri dishes with aluminum covers 1, 2.5, 7, and 27 h after fertilization and exposed to the appropriate selenium concentrations. These time intervals corresponded to the two to eight cell ovum (Stages 3–5), late blastula (Stage 10), blastoderm enveloping yolk (Stage 15), and initiation of heart beat (Stage 21) of the normal embryonic development of this species as described

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TABLE 1. Hatching of zebrafish (*Brachydanio rerio*) embryos exposed to different selenium concentrations at several stages of development. The values shown represent the geometric mean of 10 replicates for each treatment and of 62 replicates for the controls.

Selenium concn	Stage of development when exposed	% Hatch, hours after fertilization				
		≤72 h	96 h	120 h	144 h	≥168 h
0.5 μg/ml	1 h	0	3	36	31	10
	2.5 h	2	8	50	19	4
	7 h	0	3	41	32	8
	27 h	0	2	36	28	12
1 μg/ml	1 h	0	3	53	25	10
	2.5 h	0	4	41	36	8
	7 h	<1	6	41	21	9
	27 h	0	4	55	16	7
3 μg/ml	1 h	2	12	43	26	7
	2.5 h	2	10	45	15	4
	7 h	<1	8	38	24	8
	27 h	2	12	36	12	3
5 μg/ml	1 h	<1	3	47	32	6
	2.5 h	0	3	49	25	6
	7 h	0	5	41	34	6
	27 h	0	3	52	25	10
10 μg/ml	1 h	<1	4	35	24	9
	2.5 h	0	<1	54	30	9
	7 h	0	4	46	22	9
	27 h	1	3	51	17	12
Control		<1	5	47	22	7

by Hisaoka and Battle (1958). A fifth group of larvae, ranging in age from 2 to 18 h after hatching, were also exposed to selenium. There were 10 replicates for each treatment.

Selenium concentrations of 0.5, 1, 3, 5, and 10 μg/ml were prepared using selenium dioxide and reconstituted water. The water was derived by passing domestic water through an activated charcoal filter, a mixed-bed demineralizer, and a glass wool filter. The pH was adjusted to 7.0 and the total hardness to 45–50 mg/liter (as $CaCO_3$) according to Marking and Hogan (1967). Dissolved oxygen levels ranged from 6.6 to 6.9 mg/liter. Water temperature was 26 C. Selenium content was determined fluorometrically according to Lott et al. (1963). The excitation and emission wavelengths used were 405 and 535 nm, respectively. Less than 5% of the selenium in solution at each concentration was either lost or taken up by the embryos and larvae.

Dead eggs and larvae were removed daily and the media changed. The hatched larvae were maintained in the same selenium concentrations and were denied food during this period. Criteria for death used were the development of an opaque coloration by the embryos and a failure of larvae to respond to tactile stimulation. Those manifesting an extremely weak response to the touch stimulus at the end of the observation period (less than 15% of the total mortality) were also counted as dead.

Hatching and mortality among embryos were expressed as a percentage of the sample size and larval mortality as a percentage of the larvae hatched, after adjustment for mortality among the controls. A skewed distribution, similar to that observed for the natural embryo mortality and larval hatching for this species (Niimi and LaHam 1974), required that a geometric mean be calculated for each treatment. The cumulative percentages of a geometric mean do not necessarily total 100%. The mortality of embryos that died during the first 24 h of exposure and those that died before hatching, or did not hatch during the observation period, were calculated for each treatment. Mortality among the controls as well as those treated, sharply increased 312 h after hatching. This was probably attributable to factors such as food deprivation. Because the influence of such factors is not consistent with the objectives of this study, the results beyond 240 h were not included. Attempts to extend the period of observation by feeding the newly hatched larvae infusoria were not successful. Fouling of the media by dying organisms increased larval mortality.

Results — The hatching of zebrafish embryos was not affected by exposure to selenium concentrations of 0.5–10 μg/ml. Approximately 40–50% of the embryos of all groups hatched 120 h after fertilization, an additional 20–30% hatched during the following 24 h (Table 1).

Table 2. Mortality of embryos and larvae of zebrafish exposed to different concentrations of selenium at several stages of development. The values shown represent the geometric mean of 10 replicates for each treatment. Mortality rate among the embryo and larval controls was generally less than 4 and 1%, respectively.

Selenium concn	Stage of development when exposed	Embryos		Larvae			
		% Mortality after 24 h exposure	% Mortality[a] at hatch	% Mortality after hatch			
				24 h	96 h	168 h	240 h
0.5 μg/ml	1 h	<1	2	0	<1	<1	<1
	2.5 h	<1	<1	0	<1	<1	<1
	7 h	0	<1	0	0	<1	<1
	27 h	<1	<1	<1	<1	<1	<1
	Larva			0	1	1	1
1 μg/ml	1 h	<1	2	0	0	<1	<1
	2.5 h	0	<1	<1	<1	<1	<1
	7 h	<1	<1	0	<1	<1	<1
	27 h	<1	1	0	<1	<1	<1
	Larva			0	<1	<1	1
3 μg/ml	1 h	<1	2	0	6	58	96
	2.5 h	2	2	0	8	59	90
	7 h	<1	3	0	6	55	94
	27 h	1	1	1	8	53	96
	Larva			0	<1	11	29
5 μg/ml	1 h	<1	2	<1	8	54	96
	2.5 h	<1	3	<1	8	49	93
	7 h	<1	1	2	5	70	94
	27 h	0	2	<1	4	52	90
	Larva			<1	1	19	85
10 μg/ml	1 h	<1	2	<1	33	88	97
	2.5 h	0	3	<1	24	87	100
	7 h	<1	2	<1	26	90	99
	27 h	<1	2	<1	45	89	98
	Larva			<1	7	72	98

[a] Includes viable embryos not hatched during the period of observation.

Mortality among embryos exposed at different stages of development was not appreciably influenced by increasing selenium concentrations. Generally, less than 3% of the embryos exposed either died, or did not hatch, during the period of observation (Table 2). Following hatching, mortality sharply increased at concentrations of 3 μg/ml or greater. The mortality after hatching was dependent on the selenium concentration and the duration of exposure. Larval mortality after 96 h exposure at concentrations of 3 and 5 μg/ml was less than 10%, increasing thereafter to approximately 50% at 240 h. The mortality at 10 μg/ml increased at a faster rate, approaching 90% at 168 h exposure and 99% at 240 h. Concentrations of less than 3 μg/ml did not influence larval mortality.

A pronounced enlargement of the abdominal region was the only anomaly consistently observed. This condition was demonstrated by approximately 40% of the larvae at death and was first indicated by a convex flexure of the vertebral column following which there was a gradual distension of the abdominal region. Death usually occurred within 2–3 days. The frequency of this condition did not appear to be influenced by the selenium concentration or the stage of development when exposed.

Discussion — The toxicity of selenium at concentrations of 3 μg/ml or greater determined for young zebrafish is in agreement with the toxic level of 2–5 μg/ml for adult goldfish (*Carassius auratus*) reported by Ellis et al. (1937). A 7-day LC_{50} value of 12 μg/ml determined for goldfish (Weir and Hine 1970) would also be consistent with these observations. These toxic concentrations are well above most of the selenium levels of the ground and surface waters in the United States cited by Larkin and Davidson (1967), and the water quality standard of 10 μg/liter for selenium established by regulatory agencies (United States

Public Health Service 1962; Environment Canada 1972).

The newly hatched larvae were the most susceptible to selenium toxicity of all the life stages examined in the present study. This is consistent with similar observations on the effects of other elements such as zinc (Skidmore 1965), and mercury (Akiyama 1970). No suitable explanation can be suggested for the viability of zebrafish embryos in selenium concentrations up to 10 μg/ml although 3 μg/ml was lethal to the larvae. In a similar study, Huckabee and Griffith (1974) reported selenium levels of 1–5 μg/g had no effect on the hatchability of carp eggs (*Cyprinus carpio*); however, the viability of the larvae following hatching was not examined.

A short hatching time of 4–5 days after fertilization for zebrafish did not allow this criterion to be effectively used as a reliable toxicological indicator. The present study could demonstrate no differences in hatching rates among treatments (Table 1).

The limited information presently available on selenium does not allow an accurate assessment of its biochemical role in the aquatic environment. However, the accumulating information on selenium does suggest its actions may be analogous to that of mercury. There is evidence that does suggest an accumulation of selenium by aquatic organisms. The selenium levels in the muscle of Great Lakes fishes (Pakkala et al. 1972) is considerably higher than the selenium levels of the waters in the Great Lakes region (Goulden and Brooksbank 1974). Furthermore, the uptake of selenium by phytoplankton, zooplankton, and fishes has been demonstrated (Sandholm et al. 1973), and, more importantly, the methylation of inorganic selenium salts into organoselenium compounds by microorganisms has been established (Fleming and Alexander 1972). These similarities with mercury would strongly suggest a greater toxicity of selenium in an organic state than as an inorganic salt, and the probable existence of a selenium cycle in the aquatic environment. Studies on the toxicity of selenium in its different biochemical states would be desirable for its confirmation. Further studies on its distribution in and rates of uptake by aquatic organisms would be necessary before the schematics of a cycle can be suggested.

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Tocopherol Composition of Frozen Atlantic Herring (*Clupea harengus harengus*) Tissues and Oil

F. R. ENGELHARDT[1]

Department of Zoology, University of Guelph, Guelph, Ont.

J. R. GERACI

Department of Pathology, Ontario Veterinary College, Guelph, Ont.

AND B. L. WALKER

Department of Nutrition, University of Guelph, Guelph, Ont.

ENGELHARDT, F. R., J. R. GERACI, AND B. L. WALKER. 1975. Tocopherol composition of frozen Atlantic herring (*Clupea harengus harengus*) tissues and oil. J. Fish. Res. Board Can. 32: 807–809.

The concentrations of tocopherols in frozen stored Atlantic herring (*Clupea harengus harengus*) tissues were examined and found to vary with tissue type and the reproductive state of the fish. Only oc-tocopherol was found to be present in herring muscle and oil.

ENGELHARDT, F. R., J. R. GERACI, AND B. L. WALKER. 1975. Tocopherol composition of frozen Atlantic herring (*Clupea harengus harengus*) tissues and oil. J. Fish. Res. Board Can. 32: 807–809.

Nous avons étudié la teneur en tocophérols de tissus de hareng atlantique (*Clupea harengus harengus*) entreposé à l'état congelé et constaté qu'elle variait selon le type de tissu et la condition des gonades. Nous n'avons trouvé que l'octocophérol dans le muscle et l'huile de hareng.

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ATLANTIC herring (*Clupea harengus harengus*) is a food fish used for human consumption. It is also a major food in many captive marine mammal diets. As such, it is fed either whole, eviscerated, or as fillets, and almost always after lengthy frozen storage (Keyes 1968). In addition, processed herring oil may be used in nursing marine mammal diets, as well as in diets for terrestrial animals and fish.

The fats of marine fish used in animal diets are high in polyunsaturated fatty acids (Lovern 1962; Stansby 1962), and such diets generally require a balanced tocopherol polyunsaturated fatty acid ratio to ensure nutritional adequacy. As an ancillary part of a nutritional investigation on seals, a brief study was undertaken to assess the concentrations and distribution of tocopherols in the tissues of herring which had been in frozen storage for an extended period, as well as in processed oil.

Methods and materials — Whole Atlantic herring (*Clupea harengus harengus*) were used, which had been individually glazed and packaged in plastic-lined waxed cardboard boxes and were intended for human consumption. All fish used were stored 4–5 mo at −20 C. After this time, the fish were thawed in air and examined for tocopherol distribution in body muscle and gonadal tissue. Fish used for analysis were from different lots, but of uniform size, approximately 25 cm in length. Muscle samples were always taken from the same area, just ahead of the dorsal fin.

Isolation and identification of tocopherols in herring body muscle and herring oil (oil obtained from Lipman Marine Product Inc., Gloucester, Mass.) was carried out by gas–liquid chromatography, adapting methods given by Nair and Luna (1968) and Slover et al. (1969). Muscle was homogenized in redistilled ethanol with pyrogallol, followed by saponification under reflux with KOH. An internal standard was added and extraction was carried out with petroleum ether. The extract was evaporated and redissolved in benzene. The benzene solution was separated by TLC. The tocopherols were identified under UV light, scraped from the plate, and ether extracted. After evaporation of the ether, the residue was sililated. Gas chromatography of the solution was in a 3.7-m long by 3-mm ID (inside diameter) glass column packed with 0.5% Apiezon-L, 100–200 mesh on Gaschrom-Q. Flame ionization detectors were used.

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[1] Present address: Department of Biology, Southeastern Massachusetts University, North Dartmouth, Mass. 02747, USA.

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Oil was treated similarly, except that no homogenization was used, but a solution of oil in ethanol with pyrogallol was saponified directly. Three samples of each were analyzed.

Five samples each of light muscle from ripe and from nonreproductive fish, of testis and ovary from ripe fish, and four samples of dark muscle from nonreproductive fish were analyzed for tocopherol concentration of fluorometry. This used a modified method for total free tocopherols by Duggan (1959), with a combined homogenization and extraction procedure carried out under N_2. Fluorescence was measured at 340 nm, following excitation at 295 nm, using interference filters. An internal standard was used to account for any aberrations due to the presence of vitamin A or other compounds (Duggan et al. 1957). Statistical significance of the experimental results was assessed at the 95% level using *t*-tests.

Results — Gas–liquid chromatographic analysis of tocopherols in herring light muscle showed that only α-tocopherol was present. This was at a concentration of 1.7–2.1 mg/100 g of wet muscle tissue. Tocopherol in herring oil was also of the α-form, at a concentration of 11.8–14.7 mg/100 g.

Total free tocopherol concentration in light muscle of the thawed stored herring varied significantly with the reproductive state of the fish. Light muscle of ripe fish had an average concentration of 2.6 ± 0.8 mg/100 g wet weight, whereas that of nonreproductive herring with undeveloped or spent gonads was 1.4 ± 0.5 mg/100 g. Dark muscle was not significantly different from light muscle at a concentration of 2.3 ± 0.9 mg/100 g. Both gonadal tissues had a concentration significantly greater than that of any muscle examined. Testis at 5.5 ± 1.8 mg/100 g and ovary at 4.8 ± 1.3 mg/100 g were similar in tocopherol concentration.

Discussion — The presence of only α-tocopherol in herring muscle and oil supports findings by Pennock et al. (1959) in the Atlantic cod (*Gadus morhua*), Braekkan et al. (1963) in herring liver, and in other marine fish species examined by Ackman and Cormier (1967). Tocopherol concentrations in the muscle tissue of stored herring examined in this study were higher than those determined for muscle of cod and winter flounder (*Pseudopleuronectes americanus*), and similar to those of the Atlantic mackerel (*Scomber scombrus*) (Ackman and Cormier 1967). Differences in muscle tocopherol concentration, both within and between species, are apparently a function of fat content. Lovern (1962) and Stansby (1962) reported that herring muscle is relatively more fatty than either cod or flounder. Seasonal changes in herring muscle fat content also occur. Lipid analyses of the tissues described here may bear out this relationship.

It is noteworthy that the gonadal tissues of herring were found to contain high levels of tocopherol. This points out the advantage of feeding whole herring in captive diets, particularly if the fish is ripe, with large gonads that can be an appreciable part of total fish weight.

Concentrations of tocopherol in fish tissues fall markedly in the course of frozen storage (Ackman and Cormier 1967; Ikeda and Taguchi 1966). Similar findings have been reported in human adipose tissue, in which storage at −20 C caused a decline in tocopherol concentration to about 50% of original levels in 6 mo (Dju et al. 1958). Thus, the values determined for frozen stored herring tissues are bound to be lower than in the live fish. This suggests that supplementation may be useful in order to provide captive marine mammals and other species receiving marine fish as food with an adequate dietary intake of α-tocopherol, similar to one that they may have available in the wild.

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Effects of Saline Water from North Dakota Lakes on Survival of Fathead Minnow (*Pimephales promelas*) Embryos and Sac Fry

B. L. BURNHAM[1] AND J. J. PETERKA

Department of Zoology, North Dakota State University, Fargo, N.D. 58102, USA

BURNHAM, B. L., AND J. J. PETERKA. 1975. Effects of saline water from North Dakota lakes on survival of fathead minnow (*Pimephales promelas*) embryos and sac fry. J. Fish. Res. Board Can. 32: 809–812.

Eggs and sac fry of the fathead minnow (*Pimephales promelas*) were subjected in the laboratory to various dilutions of water obtained from four saline North Dakota lakes; three were sodium sulfate-, one a sodium chloride-type lake. In water from the latter, the percent of fertilized eggs surviving as sac fry for 5 days remained the same as the percent hatched: about 90% at treatment levels of 500 – 12,000 μmho and 68% in undiluted lake water of 18,000 μmho. In sodium sulfate-type water, however, about 92% of the fertilized eggs hatched at treatment levels of 500 up to only 6,000 μmho, with about 82% living as sac fry for 5 days in water from two of the lakes, and only about 54% living for 5 days in dilutions made from the most saline lake (lake water of 25,000 μmho). No sac fry survived for 5 days after hatching in sodium sulfate-type water of 12,000 μmho. From laboratory bioassays and field observations, we estimated sodium sulfate-type water exceeding 8,000 μmho during reproductive periods may decrease survival of sac fry.

BURNHAM, B. L., AND J. J. PETERKA. 1975. Effects of saline water from North Dakota lakes on survival of fathead minnow (*Pimephales promelas*) embryos and sac fry. J. Fish. Res. Board Can. 32: 809–812.

Les auteurs ont soumis des œufs et des alevins vésiculés de tête-de-boule (*Pimephales promelas*) en laboratoire à diverses dilutions d'eau provenant de quatre lacs salés du Dakota-Nord; trois de ces lacs sont du type sulfate de sodium, l'autre du type chlorure de sodium. Dans l'eau provenant de ce dernier, le pourcentage d'œufs fécondés survivant comme alevin vésiculé pendant 5 jours demeure le même que le pourcentage d'éclosion: environ 90% à des niveaux de traitement de 500–12,000 μmho et 68% dans l'eau de lac non diluée de 18,000 μmho. Dans l'eau du type sulfate de sodium, cependant, environ 92% des œufs fécondés éclosent à des niveaux de traitement de 500–6,000 μmho seulement, et environ 82% survivant comme alevins vésiculés pendant 5 jours dans l'eau des deux lacs; seulement environ 54% survivent pendant 5 jours dans des dilutions obtenues du lac le plus salé (eau de lac de 25,000 μmho). Aucun alevin vésiculé ne survécut pendant 5 jours, après incubation dans de l'eau du type sulfate de sodium de 12,000 μmho. A la suite d'essais biologiques en laboratoire et d'observations sur le terrain, les auteurs estiment que l'eau du type sulfate de sodium dépassant 8,000 μmho pendant la période de reproduction peut diminuer la survie des alevins vésiculés.

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MANY prairie pothole lakes in the upper Great Plains are high in total dissolved solids (TDS) in which sulfates often exceed carbonates (Eddy 1966; Northcote and Larkin 1966). Fathead minnows (*Pimephales promelas*) occurring in some of these lakes may be limited in others by high salinities. Fatheads were recorded in one lake in North Dakota with TDS of up to 12,000 mg liter^{-1} but died within 40 min when placed in another lake at 23,486 mg liter^{-1} (Held and

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[1] Present address: U.S. Environmental Protection Agency, Kansas City, Kansas, USA.

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Peterka 1974). Survival is also partly dependent on ion composition. In Nebraska lakes of the sodium/potassium bicarbonate type, spawning was not successful at TDS greater than 2,000 mg liter^{-1} (McCarraher and Thomas 1968), whereas in Saskatchewan lakes of the sodium sulfate types, populations of fatheads were found at TDS of nearly 15,000 mg liter^{-1} (Rawson and Moore 1944). The aim of this study was to measure the survival of fathead embryos and sac fry in waters from three sodium sulfate- and one sodium chloride-type lakes.

Materials and methods — Minnows collected from North Alkaline and South Lake George, North Dakota were held in aquaria where, under a photoperiod of 14-h daylight, eggs were spawned on the undersides of cement–asbestos pipe half-sections in 25 C dechlorinated city water with a conductivity of 480–815 μmho/cm at 25 C.

Water collected from the following lakes was filtered through 36-μ nylon screen to remove zooplankters and detritus, and stored at 10–15 C before use: (1) N. Lake George (undiluted water, 22,000–28,000 μmho), (2) N. Alkaline Lake (undiluted water 12,940–16,300 μmho), (3) S. Alkaline Lake (undiluted water, 3,640–4,400 μmho), (4) Kelly's Slough (undiluted water of the sodium chloride type, 18,000 μmho).

Salinity tolerance tests were run in diluted lake waters with conductivities of 500, 1,300, 4,000, 6,000, 12,000, and 18,000 μmho at water temperatures of 25 ± 1 C in glass test chambers, 30 × 30 × 15 cm deep. All dilutions were made with distilled water. Oxygen saturation and water circulation were maintained with air stones. A third of the water in each chamber was replaced every 2 days. Twelve hours after fertilization at least 10 eggs were transferred from aquaria to each of four baskets in each test chamber. In each polyvinyl-chloride basket (5 cm diam. × 9.5 cm deep) a shelf of 316-μ nylon screen cemented 3 cm below the rim permitted eggs to rest 1 cm below the water surface. One-centimeter holes in the baskets facilitated water circulation as determined by observing movement of methylene blue solution around the baskets. To inhibit fungal growth five drops of aqueous 1% methylene blue were added on days one and two followed by one drop every 2 days. Hatched fry were segregated daily into dated baskets in the same chamber and their survival recorded for 5 days. This was judged long enough to evaluate salinity effects on sac fry without the necessity of beginning feeding. In a trial using city water, all 123 newly hatched fry survived for 7 days without feeding.

Conductivity was determined daily and dissolved oxygen (azide modification of the Winkler method), total alkalinity, and pH every 2 days for each test. Concentrations of calcium and magnesium (atomic absorption), potassium and sodium (atomic emission), sulfate (turbidimetric method, Hach Chemical Company), chloride, and TDS (APHA 1971) were measured for each treatment replicate.

Tests of N. Lake George water were replicated 3 times and most others twice. Salinity effects were assessed by recording percent successful hatch, percent 5-day survival of sac-fry, and percent of sac fry that were normal at hatching. Abnormal fry were determined by the presence of curvature of the spine. Analysis of variance and Duncan's multiple range test were used for statistical assessment.

Results — At treatment levels ⩽ 6,000 μmho about 92% of the fertilized eggs hatched; hatching success declined to about 22% in sodium sulfate water of 12,000 μmho but remained high (98%) in sodium chloride water of 12,000 μmho from Kelly's Slough and was 73% in water of 18,000 μmho (Table 1A). At treatment levels ⩽ 6,000 μmho about 85% of the fertilized eggs lived as sac fry for 5 days, except in dilutions from N. Lake George where survival was low (about 54%) and erratic (ranging from 5 to 90%) (Table 1B). No sac fry lived for 5 days in water of 12,000 μmho or greater from N. Lake George and N. Alkaline Lake; however, in sodium chloride water from Kelly's Slough survival was 94% in water of 12,000 μmho and 68% in undiluted lake water of 18,000 μmho. A difference in response between sodium sulfate and sodium chloride water of ⩾ 12,000 μmho was also observed in regard to spinal curvature of hatched fry. In dilutions of water from Kelly's Slough of 12,000 μmho mean percent normal sac fry hatched was 96% but only 21% for N. Lake George and 24% for N. Alkaline Lake (Table 1C). Mean percent normal sac fry in water from N. Lake George of ⩽ 6,000 μmho was lower (80%) and more erratic (ranging from 50 to 100%) than was recorded in dilutions from other lakes.

In dilutions of sodium sulfate water of similar conductivity of ⩽ 4,000 μmho, amounts of HCO_3, CO_3 and Ca tended to be lowest from N. Lake George, higher from N. Alkaline Lake, and highest from S. Alkaline Lake; amounts of Cl, SO_4, Na, and Mg were similar for these three lakes (Table 2).

Discussion — We are unable to explain the apparent low and erratic survival of sac fry in dilutions of water of ⩽ 6,000 μmho from N. Lake George, as compared to survival in similar dilutions from N. Alkaline Lake where water chemistry was similar. Perhaps there are insoluble compounds in the very saline water of N. Lake George that were released when the lake water was diluted, thus producing substances toxic to sac fry. Conductivity of undiluted lake water of N. Lake George was about twice that of N. Alkaline Lake.

TABLE 1. Mean percent of A) fertilized eggs hatched, B) fertilized eggs hatching to sac fry that lived for 5 days, and C) sac fry that were normal at hatching, from various dilutions of water taken from four saline lakes, North Dakota. Numbers in parentheses are replications; ranges are given below each mean. About 40 fertilized eggs were used in each experiment.

	μmho					
Lake	500	1,300	4,000	6,000	12,000	18,000
A.) N. Lake George	90.9(3)	89.0(3)	94.8(3)	93.2(3)	30.8*(3)	
	85.1–95.0	70.0–97.5	74.0–100	84.0–97.5	20.0–31.8	
N. Alkaline	96.7(1)	97.5(2)	93.8(2)	92.5(1)	12.5*(2)[a]	
		97.5–97.5	90.0–97.5		12.5–12.5	
S. Alkaline	83.7(2)	91.3(2)	87.5(2)[b]			
	80.0–87.5	90.0–92.5	77.5–97.5			
Kelly's Slough	86.7(2)	87.5(2)	97.5(2)	93.8(2)	97.5(2)	72.5(1)
	73.3–100	75.0–100	97.5–97.5	87.5–100	97.4–97.5	
B.) N. Lake George	61.1(3)	50.8(3)	50.4(3)	54.4(3)	0.0*(3)	
	29.8–90.0	5.0–87.5	11.1–85.0	22.7–80.5	0.0–0.0	
N. Alkaline	90.0(1)	87.8(2)	72.5(2)	70.0(1)	0.0*(2)[a]	
		80.9–95.0	62.5–82.5		0.0–0.0	
S. Alkaline	82.5(2)	80.0(2)	93.9(2)[b]			
	70.0–95.0	70.0–90.0	90.-3–97.4			
Kelly's Slough	85.0(2)	87.5(2)	95.0(2)	91.4(2)	93.5(2)	67.5(1)
	70.0–100	75.0–100	92.5–97.6	85.0–97.8	89.5–97.5	
C.) N. Lake George	72.9(3)	83.3(3)	79.8(3)	82.3(3)	20.6*(3)	
	50.0–89.7	50.0–100	53.7–100	59.7–100	8.8–40.8	
N. Alkaline	93.1(1)	100(2)	94.9(2)	100(1)	24.0*(2)[a]	
		100–100	89.7–100		10.5–37.5	
S. Alkaline	85.4(2)	87.8(2)	95.5(2)[b]			
	81.3–89.5	78.4–97.3	93.6–97.4			
Kelly's Slough	97.8(2)	100(2)	97.4(2)	98.9(2)	96.0(2)	93.1(1)
	95.7–100	100–100	94.9–100	97.8–100	91.9–100	

[a] Undiluted lake water of 12,940–16,000 μmho was used.

[b] Undiluted lake water of 3,640–4,400 μmho was used.

*Statistically significant ($P \leq .05$) when compared to other means in the row as determined by Duncan's multiple range test using numerical data.

Slight decreases in dissolved oxygen with increased salinity apparently had little influence on sac fry survival as survival was good in water from Kelly's Slough where dissolved oxygen decreased by the same magnitude as in sodium sulfate-type water of similar salinity.

Although antagonistic and synergistic ion effects were not assessed, general observations were that death of sac fry in water of 12,000 μmho of the sodium sulfate type was associated with: 1) sulfates exceeding 8,000 mg liter^{-1}; tolerance limits of fatheads were 7,500 mg liter^{-1} in sodium/magnesium sulfate lakes in Saskatchewan (Rawson and Moore 1944), 2) total alkalinity exceeding 1,000 mg liter^{-1}; more than 2,000 mg liter^{-1} caused a sharp decline in spawning success of fatheads in Nebraska saline lakes (McCarraher and Thomas 1968), 3) potassium exceeding 300 mg liter^{-1}; potassium averaged 236 mg liter^{-1} (extreme of 500 mg liter^{-1} in one lake) in 16 Nebraska lakes where fatheads survived for 6 or more months (McCarraher and Thomas 1968), 4) calcium levels of 7–10 mg liter^{-1}; low calcium may increase toxicity of other chemical compounds (McKee and Wolf 1963). High pH's of 9.2–9.3 were probably not at lethal limits as pH's as high as 9.4 (mean of 9.2) were observed in N. Alkaline Lake during ice-free periods 1969–70 (Held 1971). pH's near 9.8 were tolerated by fathead minnows in Nebraska saline lakes (McCarraher 1962). Conductivity appears to be an appropriate expression of salinity for the various treatments rather than TDS. For example, in water of 12,000 μmho, millimoles per liter (osmotic concentration) were nearly equal for sodium sulfate and sodium chloride water (288 and 266, respectively), as compared to TDS of 12,098 mg liter^{-1} and 8,761 mg liter^{-1}, respectively.

Laboratory indications that fry survive at 6,000, but not at 12,000 μmho of the sodium sulfate-type water, were reinforced by field observations that waters exceeding 7,000–8,000 μmho may prevent successful reproduction. We

TABLE 2. Mean physical and chemical characteristics of various lake water solutions (μmho/cm at 25 C) for North Lake George (N.G.), North Alkaline (N.A.), South Alkaline (S.A.) and Kelly's Slough (K.S.), North Dakota. All chemical measurements are in milligrams per liter^{-1}. Means were based on three separate measurements in N.G.; two in the other lakes.

μmho	Lake	Total dissolved solids	pH	O_2	HCO_3	CO_3	Cl	SO_4	Ca	Mg	K	Na
500	N.G.	256	7.6	8.0	29	0	16	156	<1	8	9	76
	N.A.	268	6.7	7.6	51	0	27	160	<1	9	14	76
	S.A.	271	7.6	7.8	79	0	13	137	2	12	13	69
	K.S.	248	6.6	7.8	6	0	101	57	10	6	4	64
1,300	N.G.	788	8.3	8.0	72	1	45	415	1	25	16	221
	N.A.	764	8.1	7.6	149	2	44	463	1	24	31	229
	S.A.	748	8.6	7.7	192	17	31	355	3	32	26	196
	K.S.	690	6.8	7.7	11	0	285	115	25	17	7	187
4,000	N.G.	2,614	8.8	7.6	198	48	183	1,480	3	96	84	831
	N.A.	2,557	8.9	7.4	366	88	128	1,430	3	78	104	593
	S.A.	2,621	9.0	7.5	588	175	105	1,320	6	112	116	636
	K.S.	1,868	7.3	7.7	25	0	838	365	70	47	29	489
6,000	N.G.	4,869	9.0	7.4	318	102	300	3,258	4	157	146	1,333
	N.A.	4,469	9.1	7.2	566	216	230	2,900	5	135	176	996
	K.S.	3,679	7.4	7.6	33	0	1,606	810	122	96	52	943
12,000	N.G.	12,098	9.2	7.1	565	360	733	7,800	7	423	318	3,338
	N.A.[a]	13,639	9.3	6.4	1,950	902	688	8,800	10	397	537	4,075
	K.S.	8,761	7.8	7.3	66	0	3,775	2,225	275	244	120	2,640

[a] Undiluted lake water (mean of 14,600 μmho).

observed on July 14, 1973 that fathead minnow eggs spawned on floating boards placed near the shore of N. Alkaline Lake, where lake water conductivity was 8,000 μmho, had lost their adhesiveness and were easily lost from the boards; all eggs removed to the laboratory died. We observed that adult fatheads survived in N. Alkaline Lake when winter conductivities were 16,000 μmho with successful reproduction in the spring and early summer when conductivities ranged from 6,000 to 7,000 μmho.

Although laboratory experiments demonstrated that 68% of fertilized eggs hatched into sac fry in undiluted water of 18,000 μmho from Kelly's Slough, we found no fatheads in the lake, but did collect the brook stickleback (*Culaea inconstans*) and banded killifish (*Fundulus diaphanus*). We did not determine why fathead minnows were not found.

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Simplified Spectrophotometric Curcumin Method for the Determination of Boron in Marine Shellfish

R. E. Drew

Department of the Environment, Fisheries and Marine Service, Fish Inspection Laboratory, Vancouver, B.C.

Drew, R. E. 1975. Simplified spectrophotometric curcumin method for the determination of boron in marine shellfish. J. Fish. Res. Board Can. 32: 813–816.

The method involves a sulphuric acid–hydrogen peroxide digestion of the tissue sample at 100 C, dehydration of the digest with acetic anhydride, formation of the boron–curcumin complex (rosocyanin), followed by buffering of the solution and measurement of the absorbance at 545 nm. Data are presented to show that a mean relative standard deviation of approximately 5% and a mean total error of approximately 12% can be expected for shellfish in the concentration range of 1.0–10.0 μg B/g wet weight tissue (ppm).

Drew, R. E. 1975. Simplified spectrophotometric curcumin method for the determination of boron in marine shellfish. J. Fish. Res. Board Can. 32: 813–816.

La méthode comporte une digestion de l'échantillon de tissu à l'acide sulphurique–peroxyde d'hydrogène à 100 C, déshydratation du produit de digestion à l'anhydride acétique, formation du complexe bore–curcumine (rosocyanine), suivie du tamponnage de la solution et de la mesure de l'absorbance à 545 nm. Nous présentons des données démontrant qu'on peut s'attendre à un écart type relatif moyen d'environ 5% et d'une erreur totale moyenne d'environ 12% pour les mollusques, dans l'intervalle de concentrations de 1.0–10.0 μg B/g de poids de tissu humide (ppm).

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During the preceding two decades boron concentrations in domestic sewage and industrial effluents have been increasing to the point where boron could potentially become another environmental contaminant. This subject has been discussed extensively by Greenhalgh and Riley (1962), Lionnel (1970), Waggott (1969), and Wilcox (1958). More recently, the pulp and paper industry in British Columbia has made changes in the ground-wood bleaching process resulting in sodium metaborate being disposed of in the adjacent coastal waters. In view of this, it is apparent that baseline data on boron levels in coastal shellfish will be necessary for future comparison.

The number of analytical techniques that can be used to determine boron quantitatively in biological samples is limited by the fact that most are either inapplicable (e.g. neutron radioactivation, X-ray fluorescence, and polarography), previously not applied to tissue analysis (e.g. fluorometry), or insufficiently sensitive (e.g. atomic absorption spectroscopy). Among the useable techniques are reactions utilizing several types of colorimetric reagents, of which curcumin is the most sensitive and reliable (Dyrsson et al. 1972; Williams and Vlamis 1961).

A number of methods have been used to determine boron in plant materials, but few have been applied to animal tissues, and none to shellfish. In addition, available methods are further limited by the necessity of choosing a procedure in which the method of sample mineralization anticipates the possible loss of boron due to volatilization. It has been shown (Pinta 1971; Bader and Brandenberger 1968) that high temperature dry ashing of biological tissues can result in severe volatilization losses of boron, especially if the sample is rich in fats. Mair and Day (1972) pointed out that the main advantage of using a radiofrequency (oxygen plasma) biological sample asher when analyzing for boron is that temperature can be maintained within the range 100–150 C. However, the apparatus required is expensive and not readily available.

The above considerations have lead to this work which describes a simple, straightforward procedure, similar to that used by Uppström (1968) for water analysis, but developed specifically for the determination of boron in marine shellfish. The procedure incorporates a method of sample mineralization that has not previously been used in conjunction with the curcumin reagent.

Materials and methods — Apparatus — Absorbance measurements at 545 nm were made with a Bausch

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and Lomb 505 recording spectrophotometer using 1.0-cm cells.

Sample digestion and complex formation was carried out in 50-ml Vycor[1] crucibles with lids.

Reagents — a) Stock boron solutions: appropriate boron stock solutions were obtained by dissolving anhydrous H_3BO_3 in deionized water; b) curcumin–acetic acid reagent: 0.125 g curcumin (Eastman No. 1179 or equivalent) in 100 ml glacial acetic acid. This solution should not be kept for more than 2 wk; c) buffer solution: 90 ml of 96% ethanol, 180 g of ammonium acetate, and 135 ml of glacial acetic acid diluted to 1 liter with deionized water.

Unless otherwise stated, all the reagents were analytical reagent grade (Fisher Scientific). Acetic anhydride (>97%, Mallinckrodt) and oxalyl chloride (North American Scientific Chemical) were also used.

Procedure — Shucked shellfish meats are drained, blended in an Omni-Mixer or Waring Blender until homogeneous, placed in polyethylene containers, and either used immediately or frozen at −10 C until analyzed. From the homogenate, 100 ± 1 mg tissue samples are weighed into 50-ml Vycor crucibles. Likewise, 100 μl of the boron calibration standards and a 100 μl deionized water blank are pipetted into identical crucibles. To each of the standards and samples are added 2.0 ml concentrated sulphuric acid. The open crucibles are then placed in an oven at 100 C for 1 h, or until charring is complete and homogeneous solution of the tissue has occurred. After removing the crucibles from the oven, 1.0 ml of 30% hydrogen peroxide is added to the crucibles which are then gently swirled and returned to the 100 C oven for not less than 18 h. (Because traces of hydrogen peroxide interfere with the colorimetric analysis, a minimum of 18 h is essential.) At the end of this time, if the sample digests are not water white to pale yellow in color, then the treatment with hydrogen peroxide must be repeated.

When digestion is complete and the crucibles have cooled to room temperature, the samples and standards are dehydrated by sequential addition of 2.0 ml acetic anhydride, 0.20 ml oxalyl chloride, and 2.0 ml glacial acetic acid, which are mixed by gentle swirling, and allowed to stand covered for 30 min. Following dehydration, 4.0 ml of the curcumin–acetic acid reagent are added, and the crucibles again swirled. The intense purple–red solutions are covered and allowed to stand for 60 min to allow formation of the rosocyanin complex. The contents of the crucibles are then quantitatively transferred to 25-ml volumetric flasks, diluted to the mark with buffer solution, and allowed to stand 60 min for attainment of chemical and thermal equilibration. Absorbance, relative to the reagent blank, is then measured at 545 nm using 1.0-cm cells.

Results and discussion — The reaction between boron and curcumin to form the complex rosocyanin is a highly sensitive and specific reaction (Dyrsson et al. 1972). Uppström (1968) claims that the only element known to interfere directly by forming a complex analogous to rosocyanin is germanium. Thus, even if extraction with another 1,3-diol were employed (Egneus and Uppström 1973), one would still expect germanium to be a possible interferent. According to Hayes and Metcalfe (1962), the optical densities of test solutions containing 1 μg boron were unaffected by 10 mg of Be, Mg, Al, Na, K, Ca, Fe, Ti, Zr, Zn, U, PO_4^{3-} or Cl^-. Sodium, as hydroxide, caused no interferences in amounts up to 300 mg. Fluoride and nitrate both interfered with the complex formation. (The brown color formed in the presence of nitrate was thought to be the result of oxidation of the curcumin.) Spicer and Strickland (1958) consider that fluoride interferes at all levels, but according to Hayes and Metcalfe (1962) up to 5 μg of fluoride can be present in a test solution containing 1 μg of boron without changing the absorbance. The same authors show that up to 100 μg of nitrate in samples containing 1 μg of boron can also be tolerated. Pakalns (1968, 1972) found that Cr, Cu, Fe, Mg, Mn, Ni, Ti, V, and Zn do not interfere. All possible trace element interferents have not been exhaustively eliminated. However, on the basis of the studies to date, it is not unreasonable to assume that for the sample size used in this procedure, interference effects would be minimal.

The sulphuric acid–hydrogen peroxide digestion employed in this procedure is similar to the one used for the analysis of boron hydrides in biological tissues by reaction with 1,1′-dianthrimide (Soloway and Messer 1964), but has not previously been used in conjunction with the curcumin reagent. Volatilization losses are minimized by the relatively low temperature (100 C) used. It cannot be used for tissue sample weights in excess of 100 mg without increasing either the volume or the concentration of the hydrogen peroxide solution. Fortunately, the marine shellfish examined in this study naturally contain boron in sufficient quantities such that a 100-mg sample exhibits a moderate to strong colorimetric response in this procedure. Although this procedure has not been applied to freshwater shellfish, there is no apparent reason why the procedure could not be applied, except in the case of boron levels less than 1 μg/g wet weight tissue (ppm). The latter would necessitate either a larger sample size or a different procedure.

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[1] 96% silica glass (Corning Glass Works); recommended for analysis of boron (American Public Health Association 1971).

Details of the chemistry involved in this analytical procedure can be found elsewhere (Uppström 1968; Hulthe et al. 1970). However, the following points need to be made. First, as proposed by Crawley (1964), acetic anhydride was used for the water elimination step because it is a stronger dehydrating agent than propionic anhydride, which was used by Uppström (1968) and Hulthe et al. (1970). The remaining water is in a very strong sulphuric acid solution, which is hydrophilic, therefore a strong dehydrating agent is needed to react with the water even though oxalyl chloride is used to catalyze the reaction. Second, Hayes and Metcalfe (1962) and Uppström (1968) found it necessary to obtain a sulphuric acid–acetic acid ratio of 1:1 prior to the addition of the curcumin reagent. This is the purpose of adding the glacial acetic acid which, in addition, also aids in obtaining a homogeneous solution. The latter is a necessary prerequisite for the reaction to proceed smoothly to completion. Third, as found by Pakalns (1969), the solutions have to stand for 60 min to obtain full color development following dilution of the solutions with the buffer solution.

The calibration curve obtained with this procedure is linear up to a concentration of 0.020 μg B/ml in the final solution, and useable up to 0.040 μg B/ml, i.e. a concentration of 10 μg B/g wet weight tissue (ppm). The linear portion of the curve is not significantly different from those obtained by Mair and Day (1972) and Uppström (1968). The nonlinear portion is similar to the curvature encountered by Hulthe et al. (1970) when the procedure of Uppström (1968) was adapted for the automated analysis of sea water. Also, the majority of the marine shellfish that have been analyzed are in the range of 2.0–5.0 μg B/g wet weight tissue (ppm) which corresponds to the range of 0.008–0.020 μg B/ml on the calibration curve.

To obtain an estimate of the precision and accuracy for this procedure, a test sample composed of manila littleneck clams (*Venerupis japonica*) and native littleneck clams (*Protothaca staminea*) was analyzed by the method of additions on 20 different days, and the results subjected to linear regression analysis:

μg B/g added (X)	μg B/g found (Y)*	μg B/g sample (Y–X)	Least-squares estimate (y)	Absolute deviation |Y–y|
0	2.07±0.09	2.07	2.091	0.021
2.0	4.10±0.12	2.10	4.088	0.012
4.0	6.11±0.11	2.11	6.086	0.024
6.0	8.08±0.10	2.08	8.085	0.005
8.0	10.07±0.10	2.07	10.084	0.014

*Mean ± SD of 20 samples.

The least-squares estimates were derived from the best fit of the data points to the line $y = a + bx$, where y equals the μg B/g found, and x equals the μg B/g added. The least-squares estimates of a and b were 2.09 and 0.999, respectively, whereas the standard error of estimate and the sample correlation coefficient were 0.102 and 0.999, respectively. From these data it can be seen that a mean relative standard deviation of approximately 5% can be expected for marine shellfish tissue samples. A mean relative standard deviation of approximately 3% was observed for the curcumin method of Mair and Day (1972); however, their replicate samples were aqueous standard solutions and not tissue samples. This must be kept in mind when considering the mean total error value they quote.

Using the formula developed by McFarren et al. (1970) and the maximum observed deviation as an estimate of the absolute error, the total error for this procedure, over the concentration range of 1.0–10.0 μg B/g wet weight tissue (ppm), is calculated as follows:

$$\begin{aligned}\text{total error} &= \frac{\text{absolute error} + 2\,(\text{SD})}{\text{true value}} \times 100 \\ &= \frac{0.024 + 2\,(0.12)}{2.09} \times 100 \\ &= 12.2\%\end{aligned}$$

This total error is a little higher than the value of 10% which was calculated in a similar fashion by Mair and Day (1972) for their procedure, but still well within the 25% value required to classify it as an excellent method according to the criterion of McFarren et al. (1970).

To verify the "true value" of a test sample, it is desirable to choose an alternative method that utilizes a different analytical technique, and that is both precise and accurate. However, no method for the analysis of boron could be found that had a demonstrated accuracy other than recovery of boron from spiked samples. Therefore, the flame emission method of Pau et al. (1972) was chosen as the best alternative because, first, it was the only noncolorimetric method available that was sufficiently sensitive, and second, it had been compared with four procedures that used different colorimetric reagents: curcumin, quinalizarin, azomethine-H, and methylene blue. Except for the azomethine-H method, their results showed no significant bias, and the procedure was considered to be about as accurate as the colorimetric methods. They quote a relative standard deviation of about 4% for a 5-g sample of dried plant material having a boron concentration "as low as 8 ppm." Their choice of dry ashing at 500 C as the method of sample mineralization was probably necessitated by the rather large sample size required by the procedure, since wet ashing of such large samples is tedious and subject to contamination from the large volumes of acids that have to be used. Unfortunately, Pau et al. did not perform recovery experiments, and in addition, performed their comparative analyses on samples that had been previously dry-ashed. As a result, the possibility of volatilization losses was overlooked in their work.

Keeping the above in mind, the flame emission method of Pau et al. (1972) was used in an attempt to verify the true value of the test sample. To be consistent, the method was rigorously adhered to except that 20-g samples (wet weight) were used instead of 5-g samples (dry weight). The mean and standard deviation obtained for seven replicate analyses (which accounted for the remainder of the test sample) was 1.71 ± 0.32 μg B/g. This value is a little lower than the value of 2.09 ± 0.10 μg B/g, but not significantly different, and easily accounted for by volatilization losses as discussed above.

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Effects of Kokanee Salmon (*Oncorhynchus nerka*) Decomposition on the Ecology of a Subalpine Stream[1]

JEFFREY E. RICHEY,[2] MICHAEL A. PERKINS,[3] AND CHARLES R. GOLDMAN
Division of Environmental Studies, University of California, Davis, Calif. 95616, USA

RICHEY, J. E., M. A. PERKINS, AND C. R. GOLDMAN. 1975. Effects of kokanee salmon (*Oncorhynchus nerka*) decomposition on the ecology of a subalpine stream. J. Fish. Res. Board Can. 32: 817–820.

The role of kokanee salmon (*Oncorhynchus nerka*) carcass decomposition in the nutrient cycles and energy flow of a subalpine stream was investigated during a season (1970–71) of high spawner abundance and a season (1972–73) of low spawner abundance with a December storm flushing the stream of carcasses. Primary production, periphyton biomass, heterotrophic activity, and nutrient concentrations were greater downstream than upstream during the peak carcass decomposition of 1971, whereas they were the same at other times of that year and in 1972–73. Calculations showed that upstream–downstream differences in phosphate could be accounted for by the salmon carcasses.

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Nous avons examiné le rôle de la décomposition des cadavres de kokanis (*Oncorhynchus nerka*) dans le cycle alimentaire et le flux d'énergie d'un cours d'eau subalpin pendant une saison (1970–71) de grande abondance et une saison (1972–73) de faible abondance de géniteurs avec tempête en décembre qui débarrassa la rivière des cadavres. La production primaire, la biomasse du périphyton, l'activité hétérotrophe et les concentrations de substances nutritives sont plus élevées en aval qu'en amont au moment du sommet de décomposition des cadavres en 1971, alors qu'elles sont semblables à d'autres périodes de cette année-là, de même qu'en 1972–73. Des calculs démontrent que les cadavres pourraient être responsables des différences de phosphate en amont et en aval.

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THE Pacific salmon (*Oncorhynchus* sp.) spends its adult life at sea, returns to its native stream or lake to spawn, and dies after spawning. Several workers have observed that decomposition of the salmon carcasses may be important in resupplying the nursery area with nutrients (e.g. Krokhin 1967; Hall 1972). It has been suggested that the nutrients released by the carcasses may stimulate primary production (Narver 1967; Mathisen 1972).

In this report we attempt to assess the role landlocked sockeye salmon (kokanee, *O. nerka*) carcasses play in the nutrient cycles and energy flow of a subalpine stream. Evidence is presented linking changes in water chemistry and plankton production to kokanee spawning and stream flow in Taylor Creek, Calif.

Study area and methods — Taylor Creek is a short (8 km) tributary to Lake Tahoe, California–Nevada. The stream's source is Fallen Leaf Lake which spills over a small concrete dam, and it is uniform in size throughout its length with no significant feeder streams. Taylor Creek has a gradient of 4% and average flows ranging from 0.1 to 6 m^3 s^{-1}. The kokanee of Lake Tahoe spawn almost exclusively in Taylor Creek. Because of the stream's uniformity, and because the dam at the outlet of Fallen Leaf Lake blocks further upstream migration, it was assumed that any difference in upstream–downstream biological productivity or nutrient concentration might be attributed to the salmon carcass decomposition. Accordingly, phosphate, nitrate, primary production, and heterotrophic activity were monitored at monthly intervals from October 1, 1970 through September 4, 1971 at lower, middle, and upstream stations. In January, ammonia and iron were also

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[1] Contribution No. 151 from the Coniferous Forest Biome, University of Washington, Seattle, Wash. 98195, USA.

[2] Present address: Fisheries Research Institute, University of Washington, Seattle, Wash. 98195, USA.

[3] Present address: Department of Civil Engineering, University of Washington, Seattle, Wash. 98195, USA.

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measured at the three stations. In late November 1972, to further examine points of interest noted during the earlier study, we added to the above scheme samples of dissolved organic and particulate carbon, total phosphorus, total dissolved phosphorus, and periphyton biomass. This latter survey was terminated in January 1973, when there were no carcasses left in the stream.

Autotrophic carbon uptake by free-floating phytoplankton was measured with the ^{14}C-bicarbonate method (Goldman 1963). Heterotrophic activity was measured using 2-^{14}C-acetate uptake (Paerl and Goldman 1972). This method is a measure of relative heterotrophic activity. Samples were collected in the field, kept cool in the dark, and transported back to the Lake Tahoe laboratory for analysis.

Periphyton biomass was measured by scraping periphyton off rocks with a brush in a plunger and suspending the sample in filtered streamwater (Perkins 1974). An aliquot was filtered through a precombusted glass fiber (GF-C) filter, which was then analyzed for total carbon using a direct combustion technique (Armstrong et al. 1971). Free particulate carbon was measured by filtering the streamwater itself and combusting. Dissolved organic carbon was measured after the method of Menzel and Vaccaro (1964).

Water from each station was collected in polyethylene jugs, filtered, and frozen for chemical analysis. Nitrate was measured by the cadmium reduction technique (Strickland and Parsons 1968), phosphate by ammonium molybdate (Murphy and Riley 1962) with sulfuric acid used for hydrolysis of the particulate fraction, iron by bathyphenanthroline (Strickland and Parsons 1968), and ammonia by a phenolhypochlorite method (Solórzano 1969). Filtration was at low vacuum through acid-rinsed glass fiber (GF-C) filters.

Estimates of daily streamflow were obtained from the U.S. Geological Survey. Spawner abundance estimates were provided by the California Department of Fish and Game.

Results — Streamflow was about 0.5 m^3 s^{-1} in October and November, 1970 and 1972 (Fig. 1). On December 3–6, 1970, a storm occurred, producing a freshet averaging 3.96 m^3 s^{-1} with a 1-day peak of 5.71 m^3 s^{-1}. A more severe storm occurred on December 19–22, 1972, producing a freshet averaging 5.04 m^3 s^{-1} with a 2-day peak of almost 6.0 m^3 s^{-1}. These were the only storms occurring while the salmon were in the stream.

Spawner abundance was 14,000 fish in 1970 and 7000 fish in 1972. The kokanee entered Taylor Creek in September and October of both years, with the bulk of spawning occurring in November and early December. The accumulation of carcasses in the 1970–71 season reached a peak in January, with decomposition completed by the end of March. The December storm did not appear to flush the stream of many carcasses. In the 1972–73 season, there were few carcasses left in the stream after the December storm.

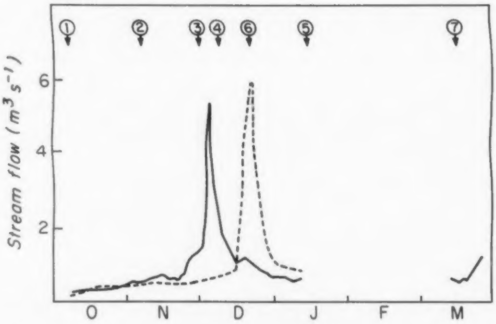

FIG. 1. Streamflow during spawning period of 1970–71 (——) and 1972–73 (---) and major events: 1) salmon entering the stream, 2) peak spawning 1972–73, 3) peak spawning 1970–71, 4) peak die-off 1972–73, 5) peak die-off 1970–71, 6) disappearance of carcasses 1972–73, 7) disappearance of carcasses 1970–71. (Flow data were incomplete, mid-January to mid-March 1971.)

Fig. 2A shows the patterns of streamborne phytoplankton carbon fixation in 1970–71 and 1972–73. In fall 1970, there was little difference between stations, with values averaging between 1 and 4 mg C m^{-2} h^{-1}. In January 1971, the time of peak carcass decomposition, there was a distinct downstream increase in productivity, with a maximum at the lower Taylor Creek station of 13.8 mg C m^{-2} h^{-1}. Over the winter months of 1972–73 there was only a small increase of downstream over upstream carbon fixation.

Coinciding with the downstream peak of productivity in January 1971 was a peak in heterotrophic activity (Fig. 2B), which extended from December through February. Heterotrophic activity at upper Taylor Creek remained very low until June, when it surpassed the other stations. This is a time of much pleasure boating and other recreational activity on Fallen Leaf Lake and its watershed; thus, the heterotrophic activity may be a reflection of increased organics in the lake water entering the stream. The downstream increase in heterotrophic activity was also observed in 1972–73 but was much less pronounced after the December freshet.

At middle Taylor Creek, and especially at Lower Taylor Creek, a very heavy, dense bloom of the periphyton *Ulothrix* occurred in January and February 1971. Unfortunately, no biomass estimate of this bloom was made. There was no difference between downstream and upstream periphyton biomass in 1972–73. Particulate and dissolved organic carbon measurements also

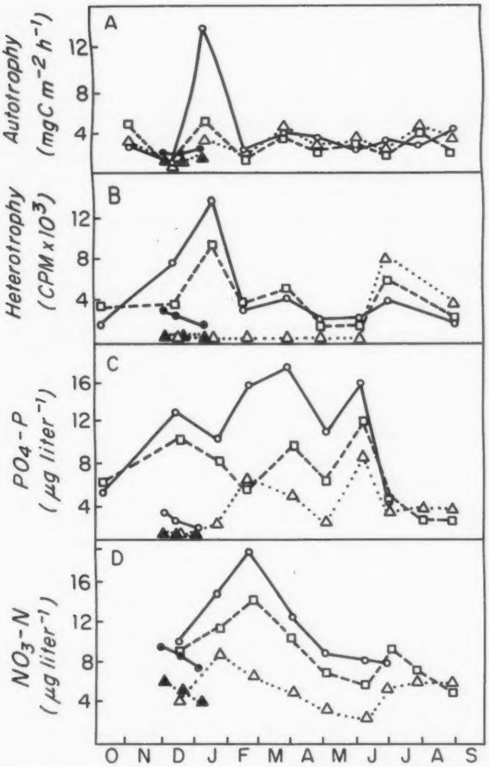

FIG. 2. Autotrophic (A) and heterotrophic (B) production of free-floating plankton, phosphate (C), and nitrate (D) concentrations. For 1970–71: Lower (○—○), middle (□---□), and upper (△....△) stations; for 1972–73: Lower (●—●) and upper (▲....▲) stations. n = 2 for (A) and (B), n = 1 for (C) and (D).

showed no downstream–upstream gradients in 1972–73.

A limnological survey of the streams of the Lake Tahoe drainage basin in 1969–70 revealed a peak in phosphate in Taylor Creek at the time of the salmon decomposition. This peak was not observed in the other, salmon-free streams of the Tahoe basin at that time (Goldman 1970). A similar nutrient pattern was evident during the 1970–71 study reported here. Phosphate concentrations (Fig. 2C) were erratic; however, downstream levels of about 12 µg liter $^{-1}$ were observed during the time of carcass decomposition, while upstream values were only about 6 µg liter^{-1}. The stream became uniform for phosphate by late spring. Nitrate concentrations increased downstream to a February maximum of 19 µg liter^{-1} (Fig. 2D). By May, Taylor Creek was relatively homogeneous with respect to nitrate at about 5 µg liter^{-1}. In January, ammonia concentrations were 6, 26, and 29 µg liter^{-1} at the upper, middle, and downstream stations, respectively. Iron concentrations were at the limit of detection of about 15 µg liter^{-1} at all stations. In 1972–73 nutrient concentrations were all lower and there was no gradient between stations.

Discussion — An approximation of the phosphorus available through the decomposition of salmon carcasses may be calculated from the streamflow and spawner abundance estimates. Assuming an average wet weight of 875 g fish^{-1}, and that body phosphorus is 0.364% of wet weight (Donaldson 1967), 4.46 × 10^{10} µg of phosphorus is contained in a salmon run of 14,000 fish. The water volume over the time of the carcass decomposition in Taylor Creek (December through March) was 1.04 × 10^{10} liters (given the average flow of 0.85 m^3 s^{-1}). Dilution of the phosphorus from the fish in that volume of water gives a final concentration of 4.3 µg liter^{-1}. The approximate difference between upstream and downstream phosphate was 6 µg liter^{-1}. Periphyton would have sequestered an unknown amount of phosphate and nitrate.

That the kokanee are indeed responsible for modifying the ecology of Taylor Creek may be inferred from the following observations. (1) An increase of phosphate in Taylor Creek coincident with the salmon run was not observed at the same time in other, salmon-free streams of the basin. (2) Carbon fixation, bacterial heterotrophy, and periphyton biomass were greatest at the peak of salmon carcass decomposition in midwinter, an unusual time for blooms. Both metabolism and nutrient concentrations were greatest downstream. The only difference noted between the upstream and downstream stations was the presence of salmon. (3) The downstream increase in nutrients and metabolism was not observed in 1972–73 when the run was smaller and a severe storm flushed the stream. (4) Calculations showed that the decomposition of salmon accounted for the difference between upstream and downstream phosphorus. (5) The presence of high downstream levels of nitrate and phosphate after the disappearance of carcasses may be due to the slow release of nutrients from the periphyton.

From the above, some aspects of the role of the kokanee salmon in the mineral cycles and energy flow of Taylor Creek might be suggested. The salmon migrate up the stream from Lake Tahoe to spawn and die. The presence of the energy-rich carcasses stimulates bacterial and fungal activity, leading to the release of nitrate, phosphate, and presumably other major and minor nutrients. The minerals released stimulate a midwinter bloom of phytoplankton and periphyton. The periphyton act as a reservoir, holding the nutrients in the spawning stream to be released after the final decay of the salmon. However, if a severe storm flushes the stream of carcasses, the nutrients will be lost and no change will be observed in the biological activity of the system.

Stimulation of bacterial and algal growth in Lake Tahoe by tributary waters is well documented (Goldman et al. 1974). Thus, nutrients released from the salmon carcasses washed into the lake during years of high spawner abundance may be important in maintaining the fertility of the nursery areas adjacent to the stream mouth where the smolts develop.

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PERSPECTIVES

Reproductive Guilds of Fishes: A Proposal and Definition

EUGENE K. BALON

Department of Zoology, University of Guelph, Guelph, Ont. N1G 2W1

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Abstract

BALON, E. K. 1975. Reproductive guilds of fishes: A proposal and definition. J. Fish. Res. Board Can. 32: 821–864.

The emergence of efficient ecosystem modelling is severely hampered by the need for an ecological classification of fishes. An earlier concept of ecological groups is here expanded to cover all living fishes. This ecological classification is based mainly on form and function in early developmental intervals, on preferred spawning grounds, and on features of reproductive behavior. Within the adaptations for reproduction and embryonic development two factors prevail — predators and the oxygen regime; consequently, spawning behavior and spawning grounds determine ensuing respiratory conditions and available protection from predators. The remaining factors are more or less associated with those two. From the point of view of resource exploitation, factors other than feeding habits governing density, biomass, and production are more important. In this sense reproductive guilds are more meaningful than feeding strategies, to which one usage of "guild" was limited.

Thirty-two guilds putatively encompass all the 30,000 living fish taxa (or about 20,000 species) and form an ecological classification unrelated to the Linnean classification. The guilds retain phyletic significance in respect to reproductive systems and form a basis for the construction of models on natural and cultural succession. Number of guilds and frequency of taxa within guilds in a given area reflect geochronological sequences and invasion abilities of members of different guilds.

Résumé

BALON, E. K. 1975. Reproductive guilds of fishes: A proposal and definition. J. Fish. Res. Board Can. 32: 821–864.

Le manque d'une classification écologique des poissons nuit grandement à l'élaboration de modèles d'écosystèmes efficaces. L'auteur se sert d'un concept existant déjà et le développe pour englober tous les poissons vivants. Cette classification est fondée surtout sur la forme et la fonction aux premiers stades du développement, sur les frayères préférées et sur le comportement reproducteur. Parmi les adaptations visant la reproduction et le développement embryonnaire, deux facteurs prédominent — les prédateurs et le régime d'oxygène; en conséquence, le comportement reproducteur et les frayères déterminent les conditions subséquentes pour la respiration et les abris disponibles contre les prédateurs. Les autres facteurs sont plus ou moins liés à ces deux-là. Du point de vue de l'exploitation des ressources, des facteurs autres que les habitudes alimentaires contrôlant la densité, la biomasse et la production ont une plus grande importance. Dans ce sens, les gildes reproductrices ont une signification plus grande que les stratégies alimentaires, auxquelles on avait limité un usage du terme « gilde ».

Trente-deux gildes englobent putativement tous les 30,000 taxons de poissons vivants (ou environ 20,000 espèces) et forment une classification écologique indépendante de la classification linnéenne. La gilde conserve une signification phylétique en rapport avec les systèmes reproducteurs et sert de base à l'élaboration de modèles de successions naturelles et culturelles. Le nombre de gildes et la fréquence des taxons au sein des gildes dans une aire donnée reflètent les séquences géochronologiques et le pouvoir d'invasion de gildes différentes.

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". . . the Coelacanth doubtless sheds its eggs inside a special case, quite possibly like those produced by some sharks and rays. Who will be the first to find one?"

J. L. B. SMITH (1957)

"Son abdomen, dilaté, recelait une vingtaine d'oeufs sphéroïdes d'un beau rouge sombre, dépourvus d'enveloppes protectrices. Leur taille égalait celle d'une orange . . ."

J. MILLOT AND J. ANTHONY (1974)

IT is generally agreed that the distribution, survival, density, and vulnerability of fish populations is limited by a complex of ecosystem factors, and a classification of fishes on an ecological basis would be most desirable. To construct an ecological organization of fishes based on the morphophyletic classification would be impossible, as the ramifications of evolution have resulted in an enormous number of morphologically distinct taxa. This is further complicated by convergence and parallelism, with the result that ecological and ethological characters are hopelessly intermingled and irretrievable at the familial and ordinal levels. An ecological classification based on reproductive strategies appears promising, however.

The difficulty in constructing an ecological classification based on reproductive strategies was well expressed by Breder and Rosen (1966): of just under 20,000 known species of fishes (Cohen 1970; Weitzman 1974) arranged in some 600 families and 30 orders (or 495 families and 62 orders according to Lindberg 1971, or 390 families, 3,713 genera, and 18,220 species according to Bailey 1971) "breeding habits" are well described for only 300 species, plus another hundred or so that are imperfectly known. Thus, any system based on reproductive strategies is, for the time being, incomplete and highly tentative. This paper should therefore be regarded as a preliminary proposal of such a system and a review of the present knowledge.

An ecological taxonomy of fishes should be simple enough to build from bits and pieces of available information about reproductive strategies. The categories should be low in number to permit ecosystem analyses, but broad enough to encompass all known species. The classification should group similar ecosystem users irrespective of phyletic origin, with the adaptations to similar ecosystems possibly reflected in convergent behavioral, morphological, and physiological characters.

My concept will be built on the basis of Kryzhanovsky's (1949) reasoning that "adaptations of fishes for spawning and development reflect not only the essential ecological factors of the embryonic period, but also the essential factors of all the other intervals of life. These adaptations mark the biology of adults, and define the type of migrations, invasion abilities, and limits of distribution" (p. 237, my translation). Some salmonids offer a good illustration. Their eggs require a high oxygen supply and, therefore, they must be hidden in the gravel of swiftly flowing streams, in clean lakes within springs, or in a substrate bathed by water currents. In the tidal zone the eggs would be destroyed by wave action and exposure; further from shore the sea bottom would provide few hiding places from the heavy predation; and at greater depths the eggs would perish from lack of oxygen. Consequently all salmonids, even those feeding in the sea or in open lakes, have to return to their special spawning grounds where the fate of the population will be decided (Kryzhanovsky 1956; Balon 1968). It is obvious that reproductive strategies *sensu lato* will be the most important characters on which the ecological classification should be built. However, if left closely related to the present morphological classification, similar ecological adaptations would be scattered throughout the present categories of more than 30 (Greenwood et al. 1966, 1967; Gosline 1971; Rosen 1973) or at best 7 "major analytic categories" (Breder and Rosen 1966) and an immense number of lower taxa. In the construction of an ecological classification a complete divorce from a Linnean classification seems to be inevitable.

Historical Review

Attempts to group fishes into other than morphological categories are only just over a quarter century old. The basic concept was, to my knowledge, formulated first by S. G. Kryzhanovsky in 1948. Although it was later repeated in detail at least twice, it somehow escaped the attention of scholars who have been active in this field. My plans 10 years later to work out a broader system with the founder never materialized.

Kryzhanovsky's (1948, 1949) hypothesis which is here used as a definition of his ecological groups has already been given in the previous section. "Two factors [writes Kryzhanovsky (1949) further] play leading roles during embryonic development: predators and availability of oxygen. All other factors are associated with these two and create together an extraordinary variety of adaptations associated with early development. However, the various reproductive strategies and spawning grounds predetermine the respiratory conditions and the potential for defense against predators. Hence, to a considerable degree, they predetermine the nature of adaptations associated with early development. Therefore, the astounding multitude of adaptations associated with development reveal the ecological patterns which reflect the essential relations of fish in nature."

To provide a better explanation of his principles for early fish development, Kryzhanovsky formulated ecological groups, all named after respective spawning grounds; for example: 1) lithophilous, 2) phytophilous, 3) psammophilous, 4) pelagophilous, and 5) ostracophilous.

All fishes belonging to the lithophilous groups deposit their eggs on a rock or gravel bottom, and on this substrate the embryos and larvae develop. This occurs in streams and rivers as well as oligotrophic lakes. Some hatch early and have a photophobic escape reaction, enabling them to scatter and hide under the stones. In this group embryonic respiratory organs are moderately developed. In all species there are segmental vessels in the ventral and/or dorsal fin fold; in some, there is also a subintestinal vein.

Fishes of the phytophilous group are adapted to develop within or on live or dead vegetation, above a muddy or silted bottom, and under conditions of very low oxygen. Larvae of this group are not photophobic and are more pigmented than the lithophils. Their embryonic respiratory organs are well, to exceptionally well, developed. They consist of the *ducti Cuvieri*, the caudal vein in the ventral fin fold, the segmental vessels in the dorsal fin fold, and in some larvae, the pectoral and gill cover vessels. Since the embryos and larvae do not hide, fecundity of the members of this group is usually very high to compensate for the high mortality caused by predators or water level fluctuations.

Some fishes, which Kryzhanovsky termed as intermediate, form a sort of transition between these two groups (e.g. *Alburnus alburnus*, *Rutilus rutilus*, *Leuciscus*, *Perca*, *Gymnocephalus*, *Stizostedion lucioperca*, *S. volgense*) and he admitted that no clear-cut division would be possible even in the best constructed system (Holčik and Hruška 1966).

Psammophilous fishes spawn on roots or grass above the sandy bottom or on the sand itself, where their embryos are adapted to develop. They are not photophobic and in contrast to phytophilous fishes, lack cement glands and cannot hang on plants. In response to moderately high oxygen availability the embryonic respiratory organs are usually limited to the *ducti Cuvieri* and the caudal vein; the gills become functional relatively late.

Fishes in the pelagophilous group scatter their eggs in the water column and the eggs are usually free floating; neutral or positive buoyancy is achieved by a very large perivitelline space and/or by numerous small oil globules or one large oil globule in the yolk. Embryos and larvae are normally not pigmented. The lack of pigment serves as a cryptic protection against predation. Embryonic respiratory organs are absent in this well-oxygenated habitat. Many of the freshwater pelagophils are of marine origin and some, such as *Lota lota*, display characteristics of a condition called semipelagophilous. One of these characteristics is a secondary loss of buoyancy in fresh water which has a lower density.

The last ecological group was called ostracophilous by Kryzhanovsky. It encompasses specialized fishes (Acheilognathinae) that deposit their eggs in the gill cavity of mussels. Not only does the adult female possess a special ovipositor for placing the eggs into the proper position in the live mollusc, her eggs are also elongated ovals that have only a barely visible perivitelline space. Consequently, more eggs can be deposited within the limited space between the gill filaments of the mussel (Balon 1962a). Embryos are especially adapted to the possibly low oxygen content of this confined environment. They have dense respiratory nets formed by the subintestinal vein on the yolk and by vessels in the dorsal and ventral fin folds. Their development is very slow and they retain the embryonic respiratory organs until they are ready to leave the mussel as advanced larvae. Prior to this, and soon after the first appearance of the eye pigment, they become extremely photophobic. This is a protective mechanism that prevents premature expulsion from the protection of the shell.

The system of ecological groups, proposed and used by Kryzhanovsky (1949; Kryzhanovsky et al. 1951), can be summarized as follows:

Fishes that scatter their eggs
- lithophilous
- intermediate
- phytophilous
- pelagophilous
- semipelagophilous
- psammophilous

Fishes that hide their eggs
- ostracophilous
- lithophilous

Fishes that guard their eggs
- lithophilous
  - adhere to the substrate in a single layer
  - in clumps
- phytophilous
- psammophilous
- nest building
  - in hollows
  - on the surface of the bottom
- brooders
- livebearers, and

fishes of unknown character (my arrangement and translation).

When this classification was applied to the members of freshwater fish families of a limited geographical area, little if any correspondence with morphological (Linnean) classification was demonstrated. Although Kryzhanovsky tried to prove that his groups were homogenous within families and with a few exceptions lacked parallelism, he also tried to prove that heterogeny with parallelism is limited to carps. However, if form and ecological function are closely related, then there should be more parallels between the ecological and Linnean taxonomy based on adult morphology. Lack of these parallels might imply that from an ecological viewpoint the Linnean taxonomy is highly artificial.

Then followed several attempts to improve the original ecological classification of fishes (Holčik and Hruška 1966; Balon 1966). Its use was extended to other geographical areas (Balon 1962b, 1964b, 1965) or applied to qualitative or quantitative values of fish populations. Basically, the latter was an extension of Swingle's (1950) concept of "balance" into a ratio of phytophils to lithophils (F/L, Balon 1962b, 1964a, 1964b), lithophils to indifferents (L/I, Holčik 1966), or biomass of lithophils to ichthyomass (L_g) and phytophil biomass to ichthyomass (P_g, Balon 1966).

To my knowledge only two independent systems were developed after Kryzhanovsky's. Breder and Rosen (1966), in an attempt to classify the data on reproduction in their giant compendium on reproduction of fishes, devised special analytic categories called "Division," seven of which encompassed all the available data. These categories "mean an assemblage of fishes that may conveniently be treated together from the standpoint of reproduction." However, their divisions retain a close relationship to the Linnean taxonomic arrangement and encompass various aspects of reproductive behavior and ecology. For example, their Division I is fairly monotypic and "the most characteristic reproductive feature of the members of this division is polyandrous mating in breeding aggregations, ... , nonadhesive pelagic eggs and virtual absence of parental care." This division consists of five primitive teleost orders. Division II, conversely, encompasses only one order, but fishes arranged in it mate in pairs or small polyandrous groups, eggs are mostly demersal and adhesive, and "may be buried, cast loose, or, in one case, actually laid out of water. Internal fertilization accompanied by an oviparous habit is reported. Parental care extends from none to cases of elaborate nest building or the carrying of the eggs in the mouth or adherent to the ventral surface of the body." Division III, which encompasses two orders, is in its reproductive characteristics very similar to Division I. Division IV includes the single order Gasterosteiformes which comprises fishes that "possess a variety of spectacular reproductive habits." Some of these habits include parental care in nests or brood pouches or hiding of eggs in the peribranchial cavity of ascidians. Breder and Rosen's system displays in some orderly fashion "trends in reproductive activities that are associated with morphology or phylogenetic relationship," but does not provide a basis for an adequate ecological grouping of fishes. However, my reorganization of Kryzhanovsky's concept was considerably facilitated by the information compiled by Breder and Rosen (1966).

A recent attempt by Nakamura (1969) to group fish ecologically is again based on early development. His categories A to F are based mainly on the spawning grounds and breeding behavior of Japanese cyprinid fishes. From the description given, his categories are similar to Kryzhanovsky's ecological groups and he quotes at least one of Kryzhanovsky's studies. Category A more or less equals phytophils, B has no equivalent in Kryzhanovsky's groups but is equal to my guarding speleophils (see later), C = lithophils, D = ostracophils, E = pelagophils, and in F, Nakamura arranges fishes of unknown spawning habits.

The behavioral groups of cichlids described by Wickler (1962, 1966) or by Fryer and Iles (1972) will also have some application in the following ecological classification. However, the reasons for not using Wickler's classification of "concealment" and "open" breeders are self-evident (see also Barlow 1974). The same applies to familial groups of Mochek (1972). A close relationship is to be remembered between guarding speleophils and bearing mouthbrooders, as illustrated by *Pelmatochromis taeniatus*, which starts off by guarding the eggs in a hole "but completes it by mouth brooding" or by *Geophagus jurupari* which has similar habits.

In the second edition of Webster's New International Dictionary of the English Language (1960) the ecological meaning of guild is defined as "an ecological group of plants distinguished from the ordinary herbs, shrubs, and trees by a special mode of life. ... The species of each of these, though unrelated, have specialized and sometimes similar physiological requirements."

The term guild was applied by Root (1967) in an attempt to arrange bird niches into "groups of species having very similar ecological roles within a community." Root wrote that "A guild is defined as a group of species that exploit the same class of environmental resources in a similar way." This can be interpreted that the primary characteristic for classification is the diet. But he implied, for *Parus inornatus*, that "while belonging to the foliage-gleaning guild with respect to its foraging habits, [it] is also a member of the hole-nesting guild by virtue of its nest-site requirements." The diet concept alone may be incomplete even in bird ecology, since Root then states "that the population's initial breeding effort will be concentrated in most favorable habitats" (p. 342). In short, the guild concept as formulated by Root (1967) may be congruent with my extended concept of Kryzhanovsky's ecological groups. In higher vertebrates the determining characters can nevertheless differ from those of fishes by reflecting the evolutionary pattern of morphological adaptations replaced by behavioral adaptations, in the same way as "the critical dimensions of the niche may be not food resources but other limiting factors" (Whittaker et al. 1973). In this sense, guilds should group quantitatively "species' niches."

According to Kryzhanovsky's hypothesis, the adaptations for spawning and early development are reflected in the biology (form and function) of adults, including their feeding habits. Therefore, the reproductive strategies of fishes may better define the ecological guilds. A good example is the case of *Alestes lateralis* in Lake Kariba. This species invaded the lake from above Victoria Falls and in a very short time replaced the abundant and indigenous *Brachyalestes imberi*. *Alestes lateralis* became the most abundant species in the lake in less than two years and exactly at a time when the reproductive conditions changed unfavorably for *B. imberi* and in favor of *A. lateralis*. The same food resources are used by both species (Balon 1971a, 1974). It is obvious, at least in fishes, that the successful existence of a certain guild depends on spawning grounds, predators, and oxygen and that these are resources that are exploited in a way similar to the universally recognized food resource.

Barlow (1974) accepted feeding guilds in Root's sense, admitting at the same time that "in both the cichlids and surgeon fishes, nonetheless, the social systems are seen ultimately as consequences of their feeding behavior, but other factors have a profound and sometimes more proximate influence. In this paper, the physical environment and the effect of predation were seen as the most important proximate modulators of the reproductive behavior in the Cichlidae." In surgeon fishes "the evidence suggests a range of reproductive behavior from group spawning through harem formation and leks, and even enduring pairs. There are some suggestions of ecological correlations with breeding strategy, but the relationships are not clear." This can merely mean that the densities and ecological production of pelagophils, "the largest single category of fishes in Hawaii" (Gosline in litt.; Jones 1968) for example, is regulated within this guild by the timing of spawning and reproductive behavior. This can be "viewed as an adaptation to avoiding egg predators" (Barlow 1974), and consequently determine acceptable densities, while it is doubtful whether the diversity of feeding habits and habitats can limit densities effectively.

"Ecological" grouping of fishes by Lowe-McConnell (1964) and Postel (1973) was limited to distribution and habitats and has no application in the present concept. The same applies to the "ecological" groups of Papadopol (1970) which were based on sexual maturity, fecundity, and egg laying frequency.

Ecological Classification

None of the previous systems fulfill the requirements called for in the Introduction, yet all provided useful information and insights. I have retained the nomenclature essentially as proposed by Kryzhanovsky and have extended it to encompass all living fishes. The Greek prefixes refer mainly to the character of usual type of spawning grounds, though other factors, such as breeding behavior, morphology of early respiratory organs, type of respiratory pigments, and behavior of embryos and larvae, are equally important in the definition of a given guild.

Thirty-two guilds putatively encompass all the living forms of fishes and form an ecological classification not related to the Linnean classification, although the current generic and specific names as well as the names of lower taxa had to be retained. The guilds are grouped into three major sections, each of which has two subsections. These are rather closely related to topics of reproductive behavior and define the ethological part of the guilds (Table 1). Hence, for example, there will be an A.1.3 guild of nonguarding, open substratum spawning lithophils and a B.2.1 guild of guarding, nest spawning lithophils.

The way the sections and their guilds have been arranged seems to indicate a direction for the evolution of reproductive details. Furthermore, diversity of guilds decreases gradually with the increased specialization. I shall return to this aspect in the discussion.

CHARACTERISTICS OF GUILDS

Only the most basic characteristics of each guild will be mentioned. For brevity, examples will be limited to freshwater fishes of Canada and

TABLE 1. Ecoethological guilds of fishes.

Section			
Subsection		Guild	
	A. Nonguarders		
A.1	Open substratum spawners	A.1.1	Pelagophils
		A.1.2	Litho-pelagophils
		A.1.3	Lithophils
		A.1.4	Phyto-lithophils
		A.1.5	Phytophils
		A.1.6	Psammophils
A.2	Brood hiders	A.2.1	Lithophils
		A.2.2	Speleophils
		A.2.3	Ostracophils
		A.2.4	Aero-psammophils
		A.2.5	Xerophils
	B. Guarders		
B.1	Substratum choosers	B.1.1	Lithophils
		B.1.2	Phytophils
		B.1.3	Aerophils
		B.1.4	Pelagophils
B.2	Nest spawners	B.2.1	Lithophils
		B.2.2	Phytophils
		B.2.3	Psammophils
		B.2.4	Aphrophils
		B.2.5	Speleophils
		B.2.6	Polyphils
		B.2.7	Ariadnophils
		B.2.8	Actinariophils
	C. Bearers		
C.1	External	C.1.1	Transfer brooders
		C.1.2	Forehead brooders
		C.1.3	Mouth brooders
		C.1.4	Gill-chamber brooders
		C.1.5	Skin brooders
		C.1.6	Pouch brooders
C.2	Internal	C.2.1	Ovi-ovoviviparous
		C.2.2	Ovoviviparous
		C.2.3	Viviparous

Europe, or, in guilds with no representatives in those areas or only with rare forms, to other fishes whose reproductive characteristics, developmental characteristics, and breeding habits are known. The availability of current catalogues (Scott and Crossman 1973; Ladiges and Vogt 1965; Banarescu et al. 1971) facilitated the selection. The European fishes, however, are given in a corrected version of Banarescu et al., based mainly on my own studies. For Canadian fishes the data compiled by Scott and Crossman (1973) were used mainly for the allocation of species into guilds. The work of Breder and Rosen (1966), especially on rare and odd species, was a constant source of inspiration and an invaluable source of data. Their text is frequently paraphrased and all unidentified quotations should be attributed to Breder and Rosen (1966).

A. Nonguarders

OPEN SUBSTRATUM SPAWNERS (A.1)

Pelagophils (A.1.1) are usually related to the sea, though sometimes only by their origin. There are, however, some known forms that are of entirely freshwater provenance like the cyprinids from the large Chinese rivers north to the Amur. Nonadhesive eggs are released and scattered in open waters, in areas where the direction of the water current is favorable to their distribution and survival. The eggs are usually spherical or elliptical, 0.5–1.9 mm in diameter without the egg membrane and 2–6 mm with the membrane. Pelagophils produce eggs in large quantities, presumably to compensate for high mortality of the larvae (*Mola mola* was estimated to have 300 million eggs). Often the eggs develop while being carried along by the current. In rivers, pelagophils may migrate several hundred kilometers upstream to spawn.

Near-neutral or positive buoyancy is achieved in several ways. In freshwater members of this guild (i) the eggs absorb water into the perivitelline space which expands after fertilization 2–5 times its size, stretching the egg membrane and enlarging the egg size (Fig. 1a, 2a) accordingly, or, (ii) water is absorbed into the egg membrane which swells into a gelatinous substance leaving the perivitelline space relatively small. This gelatinous mass of many eggs forms a compact veil in which the eggs are embedded, and the whole structure then floats like a raft on the surface of the sea (*Ophidium barbatum*, *Carapus*, *Scorpaena*, *Histrio histrio*, *Antennarius marmoratus*). The veils of *Lophius americanus* are often 9 m long and 80 cm wide (Agassiz 1882). Some freshwater pelagophils have a higher content of water in the yolk than the substrate spawners, a buoyancy adaptation which prevails in marine pelagophils (Fig. 2b) where the water content in the yolk reaches ~ 95% as compared to ~ 65% in eggs deposited on the bottom. In marine members of this guild, however, the perivitelline space rarely swells (Ehrenbaum 1964) and usually there is a high content of water in the yolk, an ineffective adaptation in fresh water (Stokes' law).

In both marine and freshwater pelagophils, the oil globule, which is greatly oversized in some freshwater forms (Fig. 1b), forms an additional hydrostatic organ effective in later phases of development (Kryzhanovsky 1960). Probably a centrally located cluster of oil globules (Nose 1971, 1972) causes eggs of the eel to ascend at an optimum rate. All these buoyancy adaptations frequently assure, in a most delicate way, optimal conditions for the developing eggs (Alderdice and Forrester 1971). For example "the eggs of *Argentina silus* may sink in sea water with a specific gravity of 1.02738 but float in sea water with one of 1.02870. Since surface water ... had the first-mentioned density and the deeper water was more dense, it follows that these eggs are bathypelagic under such conditions."

Instead of or in addition to the oil globule, marine species have a special sinus in the anterior part of the enlarged dorsal fin fold into which water gradually moves as the yolk is absorbed (Fig. 2c, d). In *Loweina rara* both dorsal and ventral fin folds develop into extremely large inflated and translucent sinuses (Fig. 2f), larger than in other known lanternfish larvae (Moser and Ahlstrom 1970). This sinus acts as a form of hydrostatic organ but its main function is to take up surplus water from the absorbed yolk when that water cannot be excreted through membranes, blood vessels, or excretory organs. Later when these organs develop, water can be expelled from the fin-fold sinus (Kryzhanovsky et al. 1953).

Some larvae develop special temporary appendages to achieve a higher volume to surface ratio and near-neutral buoyancy (Fig. 1d, e, 2e). Egg membranes of some exocetids, for example, are equipped with long filaments (Fig. 1c) with which the eggs can attach to a variety of floating objects (*Fodiator acutus*, *Parexocoetus brachypterus*, *Cypselurus californicus*).

Pelagophils are adapted to develop in waters well saturated with oxygen. Usually no special embryonic respiratory organs are formed or such organs, if present, are poorly developed. The blood vessels do not come near the surface of the body. As a special protection against oxygen poisoning, erythrocytes do not appear for a long time and plasma alone is driven along the vessels by heart beats. In addition, a lack of blood pigment, melanophores, and chromatophores makes the embryos and larvae less visible and, therefore, affords protection against predation. Because of the low nutrient content in the watery yolk, eggs of pelagophils have less vegetative plasma than do members of the other guilds, which in turn results in the formation of a relatively small embryo and larva. This small size, in the early stages of development, facilitates absorption of oxygen by the entire body surface. Formation of gills is nearly always delayed; in some, erythrocytes and gills are missing during the whole larval period. In eels the larval period can be extremely prolonged.

Embryos and larvae of pelagophils swim constantly. Strong phototropism keeps them in the open and away from shaded areas. Cement glands do not develop and thus relaxation is impossible. If an individual sinks, at first contact with the bottom it immediately swims up again. As a rule, pigment appears late in development and to a lesser degree than in other guilds; melanophores form a single ventral row and yellow pigment develops early in the eyes, yolk, and ventral row (Breder 1962).

The following Canadian freshwater fishes belong to this guild: *Anguilla rostrata*, *Alosa sapidissima*, *Coregonus alpenae*, *C. nigripinnis*, *C. reighardi*, *C. zenithicus*, *Notropis atherinoides*, *Aplodinotus grunniens*.

In Europe, excluding Asia Minor and the area east of the Sea of Azov, the following freshwater fishes putatively belong to this guild: *Anguilla anguilla*, *Alosa alosa alosa*, *A. a. bulgarica*, *A. a. macedonica*, *A. caspia nordmani*, *A. maeotica*, *A.*

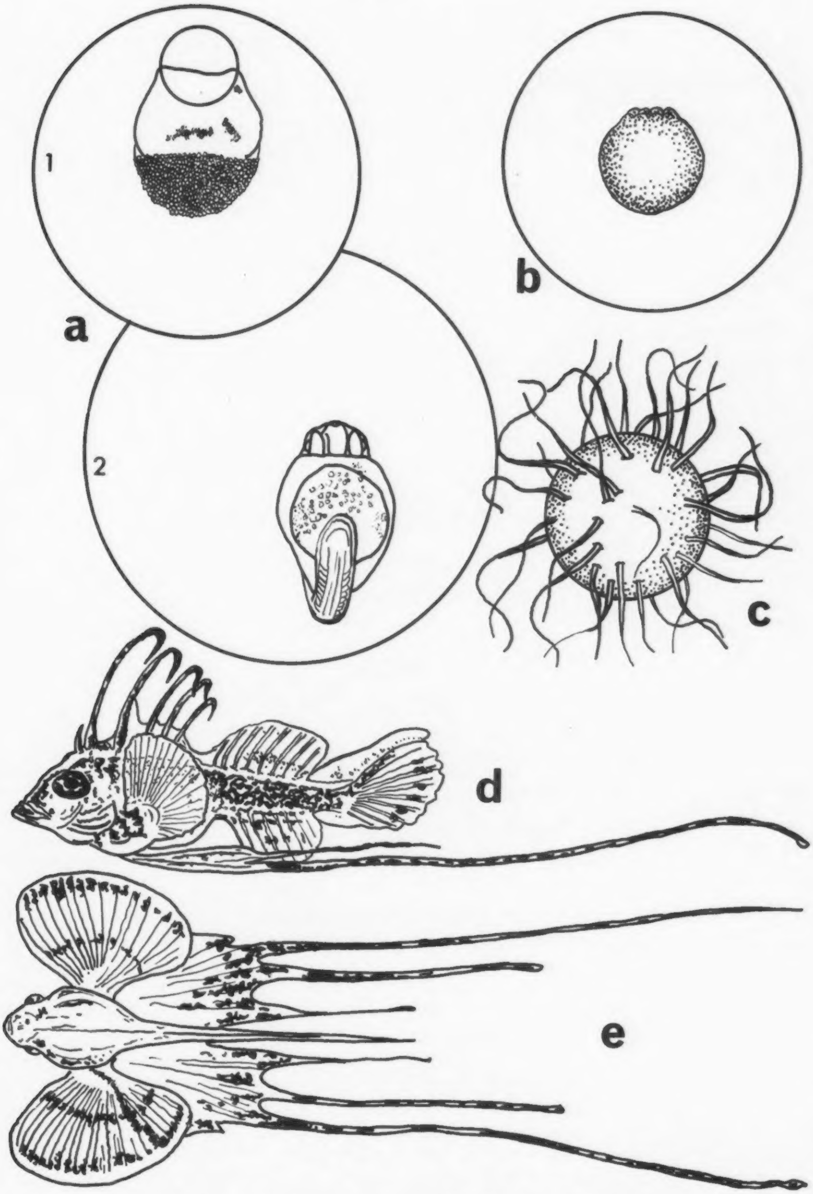

FIG. 1. a, Pelagic egg of the marine *Clupeonella delicatula* adapted for buoyancy in fresh water (diameter of yolk 0.59, of oil globule 0.32, and of egg membrane 1.3 mm); 1 = in cleavage phase, 2 = embryonic phase with increased size of perivitelline space; b, pelagic egg of the freshwater *Sarcocheilichthys sinensis* (diameter of yolk 1.9, of the egg membrane 5.3 mm); c, *Belone* sp. with tendrils for attachment to floating objects; d, e, *Lophius* sp. larva 30 mm long, lateral view (d) and dorsal view (e). After (a) Kryzhanovsky 1956, (b) Kryzhanovsky et al. 1951, (c) Bertin 1958, and (d, e) Agassiz from Ehrenbaum 1964.

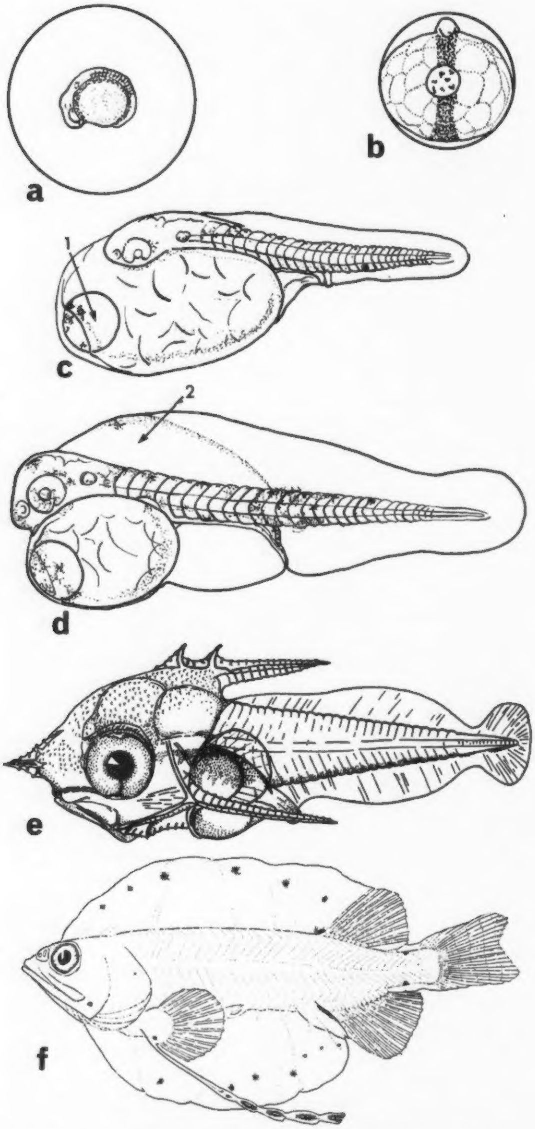

FIG. 2. a, Freshwater pelagic embryo of *Ctenopharyngodon idella*; b, marine pelagic egg of *Caranx* (watery yolk and an oil globule instead of perivitelline space); c, eleutheroembryo of *Caranx* just after hatching (1 = oil globule); d, same, at the beginning of the formation of the dorsal sinus (2); e, pelagic larva of *Adioryx vexillarius* with appendages for buoyancy; f, larva of *Loweina rara* (17.6 mm long) with dorsal and ventral fin fold sinuses. After (a) Nakamura 1969, (c, d) Kryzhanovsky et al. 1953, (e) McKenney 1959, and (f) from Moser and Ahlstrom 1970.

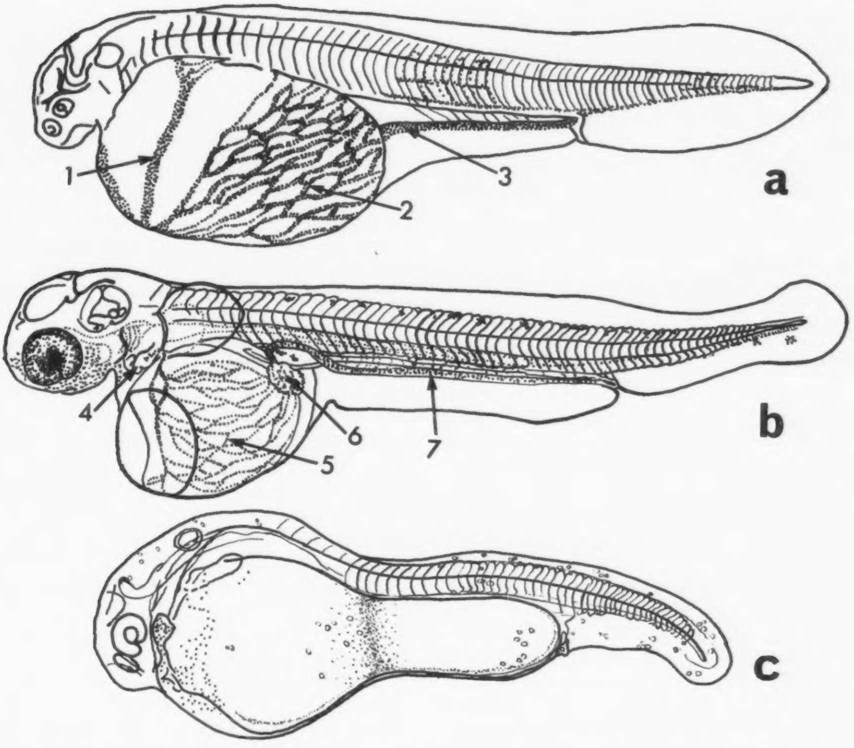

FIG. 3. a, Newly hatched eleutheroembryo of *Acipenser stellatus* 9 mm long (1 = Cuvier's duct, 2 = yolk respiratory vessels, 3 = subintestinal vein); b, eleutheroembryo of *Stenodus leucichthys* 9.4 mm long (4 = heart, 5 = respiratory branches of hepato-subintestinal veins, 6 = liver, 7 = subintestinal vein); c, eleutheroembryo of *Abramis ballerus* 3.8 mm long. After (a) Detlaf and Ginzburg 1954, (b) Smolyanov 1957, and (c) Balon 1959a.

pontica pontica, A. fallax fallax, A. f. killarnensis, A. f. lacustris, A. f. nilotica, Clupeonella cultriventris, Coregonus nasus, Pelecus cultratus, Dicentrarchus labrax, D. punctatus, Liopsetta glacialis, Platichthys flesus.

Litho-pelagophils (A.1.2) were considered by Kryzhanovsky to belong to lithophils with which they share numerous common characters. This guild encompasses fishes that go through a full range of transition from lithophils to pelagophils. Although their eggs are deposited on rocks and gravel, in some the eggs, in others the eleutheroembryos[1] and larvae become buoyant and water currents usually carry them away from spawning substrate, because they are not much heavier than the surrounding water. Sturgeon eggs adhere to the gravel and because the embryos hatch early, they are little developed but have a rich network of branches of subintestinal vein which are situated on a large yolk and form the main respiratory organ (Fig. 3a). In spite of a negative buoyancy the wide fin fold enables the embryo to make jumps towards the surface, resulting in a zigzag drift with the current. Embryos are not photophobic but there is no conclusive proof of phototropism. Some coregonids, which can also be fitted into this guild, spawn during winter in very cold waters. Their egg membranes remain slightly adhesive for only a short time; their larvae are semibuoyant and do not seek concealment on the bottom but swim immediately after hatching and display no photophobia. It is not known whether or not they are actually attracted to light. Burbot eggs are semi-buoyant due to a large oil globule in the yolk and the slightest water movement can lift them from the bottom. The eleutheroembryo looks like a typical pelagophil as it has no embryonic respiratory organs.

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[1] Eleutheroembryo = yolk-fed embryo free from egg membranes (after hatching), and larva = the following period of exogenous nutrition with numerous temporary organs (see Balon 1971b).

Canada: *Acipenser brevirostrum, A. fulvescens, A. medirostris, A. transmontanus, Dorosoma cepedianum, Coregonus artedi, C. hoyi, C. johannae, C. kiyi, Hiodon alosoides, H. tergisus, Lota lota, Morone saxatilis.*

Europe: *Huso huso, Acipenser nudiventris, A. ruthenus, A. güldenstaedti, A. naccari, A. stellatus, A. sturio, Coregonus albula, C. baunti, C. oxyrhynchus, C. peled, C. pidschian, Osmerus eperlanus, Lota lota.*

Lithophils (A.1.3) deposit eggs on a rock, rubble, or gravel bottom where their embryos and larvae develop. These sites can be in streams and rivers as well as in mesotrophic lakes. Usually embryos hatch early and are highly photophobic which helps them to scatter and hide under stones. Cement glands do not develop. Fishes of this guild are adapted to well-oxygenated waters and have moderately developed embryonic respiratory organs — *ducti Cuvieri, vena caudalis inferior* in the ventral fin fold, and later segmental vessels with loops at the base of the dorsal fin fold. In some, the subintestinal vein also functions as a respiratory organ. Black and yellow pigments appear relatively late (Fig. 3b, c).

Canada: *Coregonus autumnalis, C. laurettae, C. sardinella, C. clupeaformis, C. nasus, C. canadensis, Prosopium coulteri, P. cylindraceum, P. williamsoni, Thymallus arcticus, Osmerus mordax, Spirinchus thaleichthys, Acrocheilus alutaceus, Clinostomus elongatus, Couesius plumbeus, Mylocheilus caurinus, Notropis anogenus, N. blennius, N. dorsalis, N. rubellus, Platygobio gracilis, Ptychocheilus oregonensis, Rhinichthys atratulus, R. cataractae, R. falcatus, R. osculus, Semotilus margarita, Catostomus catostomus, C. columbianus, C. commersoni, C. platyrhynchus, Hypentelium nigricans, Minytrema melanops, Moxostoma anisurum, M. carinatum, M. duquesnei, M. erythrurum, M. hubbsi, M. macrolepidotum, M. valenciennesi, Percopsis omiscomaycus, Stizostedion canadense, S. vitreum.*

Europe: *Stenodus leucichthys leucichthys, S. l. nelma, Coregonus lavaretus, Abramis ballerus, A. sapa, Alburnoides bipunctatus, Aspius aspius, Aulopyge hügeli, Barbus barbus barbus, B. b. bocagei, B. b. plebejus, B. b. sclateri, B. albanicus, B. comiza, B. cyclolepis, B. euboicus, B. graecus, B. meridionalis meridionalis, B. m. petenyi, B. m. graellsi, B. microcephalus, B. prespensis, B. steindachneri, Chalcalburnus chalcoides, Chondrostoma nasus, C. genei, C. phoxinus, C. polylepis, C. soëtta, C. toxostoma toxostoma, C. t. arrigonis, Leuciscus svallize, L. cephalus, L. illyricus, Paraphoxinus alepidotus, P. adspersus, P. croaticus, P. epiroticus, P. ghetaldi, P, metohiensis, P. pstrossi, Phoxinellus hispanicus, Phoxinus phoxinus, Vimba vimba, V. elongata, V. melanops, Caspiosoma caspium.*

Phyto-lithophils (A.1.4) deposit eggs in relatively clearwater habitats on submerged plants, if available, or on other submerged items such as logs, gravel, and rocks. Relatively late hatching and the presence of cement glands makes some members similar to members of the next guild. Moderately developed embryonic respiratory organs and photophobia make others similar to lithophils (Fig. 4a, b).

A small controversy developed over this group when Holčik and Hruška (1966) found *Rutilus rutilus* and *Abramis brama*, originally assigned as phytophils by Kryzhanovsky and me, spawning on rocks and gravel. As a consequence, a group of indifferent (= Kryzhanovsky's intermediate) fishes was created. However, as already explained elsewhere (Balon 1966), when allocation was based only on spawning grounds, it was wrong. This should serve as a warning that for many species, arrangement into given guilds has to be tentative until there is evidence to make a categorical assertion. Holčik and Hruška's argument, nevertheless, led to the creation of this and two other, even more intermediate guilds (Table 1).

The true intermediate character of this guild can be best illustrated by the common herring. The Atlantic subspecies *Clupea harengus harengus* usually deposits adhesive eggs on a gravelly or rocky sea floor. However, there are records of eggs deposited on seaweeds, logs, and even man-made floating objects. This fish should then be considered as a true phyto-lithophil. The Pacific subspecies *C. harengus pallasi* spawns in much shallower waters than its Atlantic congener and prefers to deposit eggs on plants like *Zostera, Cystophilum, Ptilota,* and to a lesser degree on *Laminaria*, probably because of its slimy surface. Only a limited number of eggs are found on gravel or sand, and such cases are the result of violent spawning activity rather than a deliberate deposition. Some eggs are known to adhere even to living shrimps (Kryzhanovsky 1956). Therefore, this subspecies should be a phytophil rather than an ill-defined intermediate. The proper fit of this subspecies into a correct guild is further complicated by the pelagophilous structure of the embryos which have no special respiratory vessels and lack erythrocytes. The embryos' slower development, extended time of hatching, and early

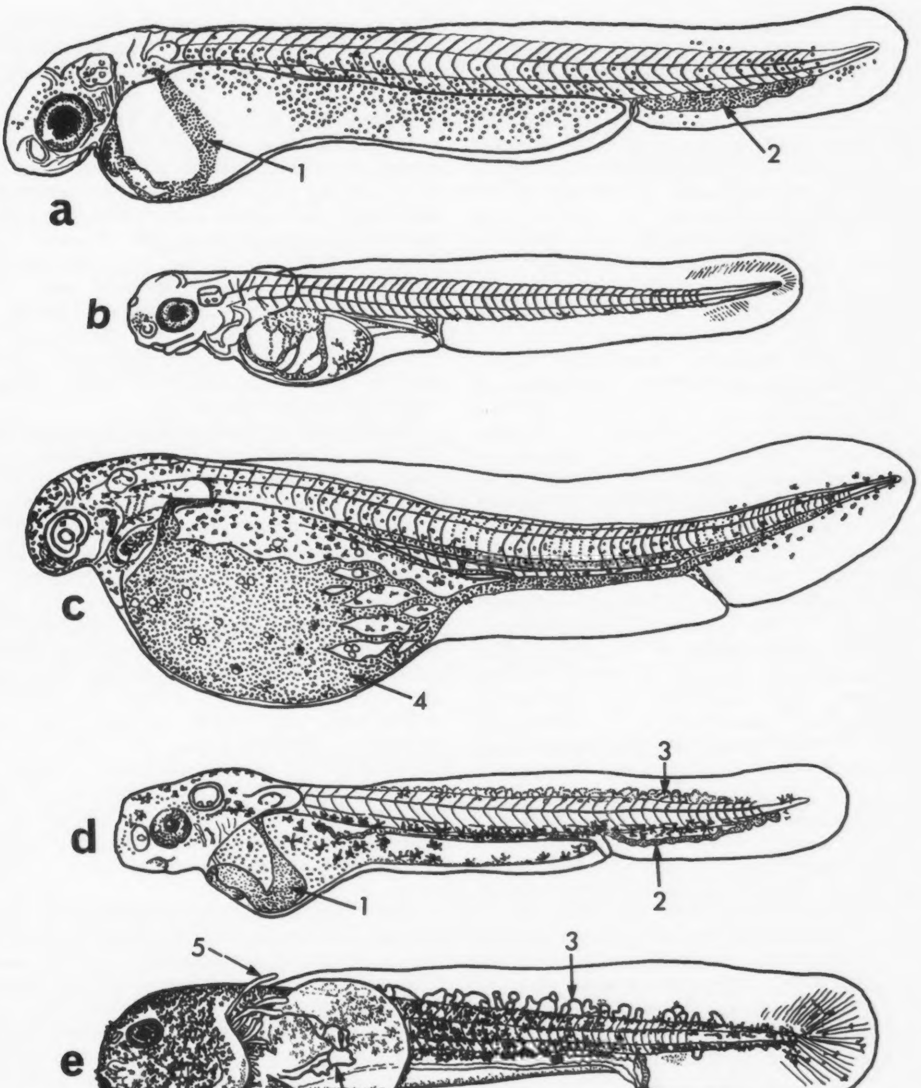

FIG. 4. a, Eleutheroembryo of *Rutilis frisi*, 8.5 mm long (1 = Cuvier's duct, 2 = lower caudal vein, points on the body surface are hatching glands); b, *Perca fluviatilis* 5.3 mm long; c, *Esox reicherti* 8 mm long (4 = respiratory organ of subintestinal vein on the yolk); d, *Cyprinus carpio* 6.7 mm long (3 = respiratory segmental vessels in dorsal fin fold); e, *Misgurnus fossilis* 5.6 mm long (5 = external gill filaments, 6 = respiratory vessels in the pectoral fin). After (a) Smirnova 1957, (b) Kryzhanovsky et al. 1953, (c) 1951, and (d, e) Kryzhanovsky 1949.

occurrence of yellow pigment (Kryzhanovsky 1956) reflects the poor oxygen conditions to which they are exposed.

The eggs of perches (*Perca* sp.) are not deposited simply but in long ribbons with a system of interior passages and a special water-pumping apparatus enhanced by the vibratory consistency of the whole egg mass. The substratum, in this case, is of little classificatory value and is more a matter of availability than selection. The same may apply to the wolffishes (*Anarhichas* sp.) whose eggs form compact balls that adhere as a whole to gravel, rocks, or plants.

Canada: *Acipenser oxyrhynchus, Alosa aestivalis, A. pseudoharengus, Hypomesus olidus, Phoxinus neogaeus, Hybognathus hankinsoni, H. nuchalis, Hybopsis storeriana, H. x-punctata, Notropis spilopterus, N. umbratilis, N. volucellus, Richardsonius balteatus, Fundulus heteroclitus, Microgadus tomcod, Labidesthes sicculus, Morone americana, M. chrysops, Perca flavescens, Etheostoma exile.*

Europe: *Abramis brama, Alburnus alburnus, A. albidus, A. charusini, Leuciscus leuciscus, L. idus, L. borysthenicus, L. microlepis, L. polylepis, L. soufia, L. tueskyi, L. ukliva, Pachychilon pictum, Rutilus rutilus rutilus, R. r. caspicus, R. r. carpathorossicus, R. r. heckeli, R. frisi frisi, R. f. meindingeri, R. f. velencensis, R. lemmingi, R. macedonicus, Perca fluviatilis, Gymnocephalus acerina, G. cernua, G. baloni, G. schraetser, Percarina demidoffi.*

Phytophils (A.1.5) scatter or deposit eggs with an adhesive membrane that sticks to submerged, live or dead, aquatic plants, or to recently flooded terrestrial plants; sometimes they deposit eggs on logs and branches but never on the bottom. Occasionally the eggs fall on stones, a type of replacement substrate where habitats have been altered by man. However, some such as most *Esox* sp. and *Cyprinus carpio* are adapted to spawn only on freshly flooded plants. If flooded vegetation is not available, spawning cannot take place.

Fishes in this guild are adapted to survive in habitats with dense plant growth and muddy bottoms and, therefore, with very low oxygen concentration. Embryos normally hatch later than those of lithophils. They have no photophobic reaction and cement glands are always present on their heads. This enables them to rest and take cover by attaching themselves to plants before they can swim actively and avoid hypoxic conditions on the bottom. Eggs and larvae are very small. In general, the postanal part of the eleutheroembryo is bent upwards to facilitate uplift and to prevent fish from falling to the bottom after hatching, before they are able to hang on plants (Fig. 4c, d). This is contrary to the larvae of lithophils which have downward-bent tails and are bottom dwellers.

In those fish that live in very poorly oxygenated waters, embryonic respiratory organs are extremely well developed (Fig. 4c, d, e), develop early, and last longer than those of embryos from well-oxygenated waters and consist primarily of the *ducti Cuvieri* and dense nets of vessels in the dorsal fin fold. The former are wide and long on the especially enlarged anterior part of the yolk and the latter last into the larval period and remain functional even at the beginning of gill function. In more highly oxygenated waters, duration of use and degree of development of respiratory organs decreases.

Canada: *Lepisosteus oculatus, L. osseus, Dallia pectoralis, Umbra limi, Esox americanus americanus, E. a. vermiculatus, E. lucius, E. masquinongy, E. niger, Chrosomus eos, Notemigonus crysoleucas, Notropis bifrenatus, N. emiliae, N. heterodon, Erimyzon sucetta, Ictiobus cyprinellus, Fundulus diaphanus, Etheostoma blennioides, E. microperca.*

Europe: *Esox lucius, Cyprinus carpio carpio, Blicca bjoerkna, Carassius carassius, C. auratus gibelio, Phoxinellus minutus, Rutilus alburnoides, R. macrolepidotus, R. pigus pigus, R. p. virgo, R. rubilio rubilio, R. r. arcasi, Scardinius erythrophthalmus, S. graecus, Tinca tinca, Cobitis taenia, C. calderoni, C. elongata, Sabanejewia aurata, S. bulgarica, S. conspersa, S. larvata, S. romanica, Misgurnus fossilis, Aphanius fasciatus, A. iberus, Valencia hispanica, Atherina hepsetus, A. boyeri, A. presbyter.*

Psammophils (A.1.6), characteristics for which are also taken from Kryzhanovsky (1949), hatch on the surface of sandy bottoms and are mainly adapted to life in running waters. Their eggs are scattered directly on the sand or near fine roots of plants that hang over the sandy bottom. In the latter case, eggs adhere to the roots and embryos fall to the bottom only after hatching. The eggs have an adhesive membrane, but no cement glands are present in embryos. Eggs of psammophils, like those of phytophils, are frequently small but the psammophils' spawning season is somewhere between that of lithophils and phytophils. The embryos become active immediately after hatching. This immediate activity facilitates emergence from the plant roots and enables them to drift towards the sandy bottom. Soon afterward all activity ceases. The embryos are phototropic which enables avoidance of crevices and other shady areas with a disadvantageous oxygen content.

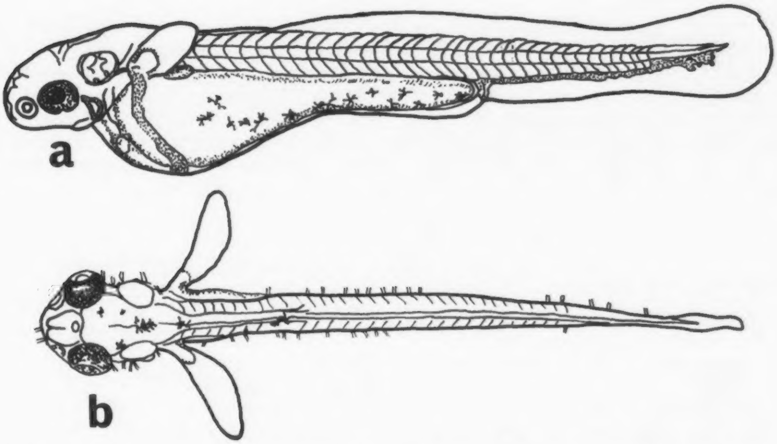

FIG. 5. a, Eleutheroembryo of *Gobio gobio* 4.7 mm long; b, the same 6.4 mm long from above (notice the sensory cupulae and large pectorals). After Kryzhanovsky 1949.

Adaptation to highly oxygenated waters is reflected in respiratory organs limited to the *ducti Cuvieri* which become shorter relatively early and to the little developed *vena caudalis inferior* (Fig. 5a). Pectoral fins appear very early and develop into large appendages that, when they are spread, enable the embryos and larvae to remain at the bottom in a vertical position (Fig. 5b). In view of this mode of life the swimbladder is reduced in size. Only in some members of this guild do the pectoral fins develop simple respiratory vessels as do the dorsal and ventral fin folds; gills also become functional early, with water entering wide-open gill apertures before the mouth is functional.

Canada: *Thaleichthys pacificus, Notropis heterolepis, N. hudsonius, N. stramineus, Carpiodes cyprinus, Catostomus macrocheilus, Ammocrypta pellucida*, and *Percina caprodes.*

Europe: *Gobio gobio, G. albipinnatus, G. kessleri, G. uranoscopus, Nemacheilus barbatulus, N. angorae, Mugil cephalus, M. auratus, M. labeo, M. labrosus labrosus, M. l. septentrionalis, M. ramada, M. saliens.*

BROOD HIDERS (A.2)

Most authors consider the preparation of redds and other similar constructions as nest building. After depositing eggs in such constructions, the fish abandon them immediately. Consequently, a subsection for nonguarding nest spawners seems appropriate. In general, however, some degree of preparation and protection of hiding places or objects hardly equals nest building and is common to all guilds in this subsection. I am inclined to consider as true nests only those places or constructions in which parental care extends beyond initial egg deposition.

Lithophils (A.2.1) of this subsection hide their eggs in natural or specially constructed places. None guard the deposited eggs, although the hiding place can be guarded throughout the spawning period, and *Oncorhynchus nerka* is known to remain with the eggs for up to 3 weeks until flushed away. In most cases the hiding places (called redds in salmonids) are excavated in gravel by the female, well in advance of the mating act. One fish can dig more than one hiding place. Generally, eggs are buried under gravel about twice the height of the body of the digging parent. In such hiding places oxygen conditions are much less favorable than on the open substrate and members of this guild are extremely limited in their choice of spawning grounds. Clean gravel or rocks and cold, clean, fast flowing water or springs are almost essential to assure at least some exchange of water around the eggs and to provide sufficient oxygen. Hidden eggs are better protected and are more likely to survive than exposed eggs. Therefore, the female can channel her reproductive energies into making fewer large eggs instead of many small ones. In Salmonidae, eggs are about 5 mm in diameter. A large, dense yolk with a membrane impermeable to water forms an enormous surface for a special embryonic respiratory organ (Fig. 6a, b). This organ is a rich network of vessels branched over the entire yolk surface (Olko 1955) between the liver and a collecting vitelline vein. Furthermore,

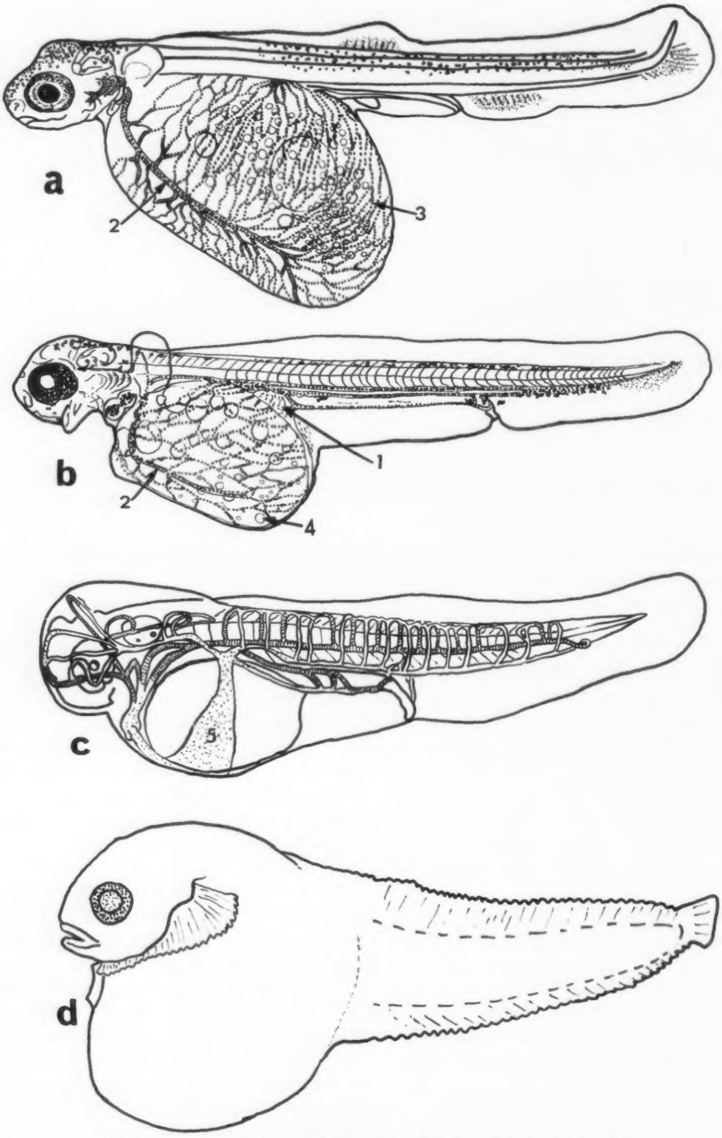

FIG. 6. a, Eleutheroembryo of *Oncorhynchus keta* 20.5 mm long (2 = vitelline vein, 3 = hepato-vitelline respiratory vessels); b, *Thymallus thymallus* 24 mm long (1 = liver, 4 = oil globule); c, *Anoptichthys jordani* 3.4 mm long (5 = Cuvier's duct); d, embryo of *Careproctus melanurus* (?). After (a) Disler 1957, (b) Balon 1967a, (c) Frank 1961, and (d) Peden and Corbett 1973.

the embryos hatch very early, and this facilitates absorption of oxygen. Since they are strongly photophobic they remain hidden under the gravel even after hatching and until most of the yolk is digested. The eleutheroembryo can roll and push itself away from the original place of incubation in a drive toward better oxygen conditions. Before eleutheroembryos emerge they disperse under the surface of the bottom. Later they emerge as large, fully formed, active alevins. This further decreases their mortality from predators.

Some, for example *Salvelinus namaycush*, dig no redds but spawn over a rocky bottom where the eggs settle in crevices and "honeycomb" cavities. This is generally considered a secondary adaptation. The eggs of most fishes in this guild are nonadhesive, but *Hucho taimen*, *Brachymystax lenok* and, to some lesser extent, *Thymallus arcticus* are reported to have adhesive eggs; this can represent an adaptation for spawning without a previously prepared hiding place for the eggs. The eggs of *Procatopus similis* or *Hesperoleucus symmetricus*, the female of which squirts her eggs into a deep crack in the rocks, are strongly adhesive. *Hybopsis micropogon* constructs redds of singly picked pebbles and after each mating adds more pebbles to cover the eggs.

Canada: *Oncorhynchus gorbuscha*, *O. keta*, *O. kisutch*, *O. nerka*, *O. tshawytscha*, *Salmo clarki*, *S. gairdneri*, *S. salar*, *Salvelinus alpinus*, *S. fontinalis*, *S. malma*, *S. namaycush*, *Stenodus leucichthys*, *Nocomis biguttatus*, *N. micropogon*, *Semotilus atromaculatus*, *S. corporalis*, *Etheostoma caeruleum*, *Percina copelandi*, *P. maculata*, *P. shumardi*.

Europe: *Salmo salar*, *S. trutta*, *Hucho hucho*, *Salmothymus obtusirostris*, *S. ohridanus*, *Salvelinus alpinus*, *Thymallus thymallus*, *Zingel zingel*, *Z. asper*, *Z. streber*.

Speleophils (A.2.2) are a peculiar guild that could have been placed in the previous subsection. Even though the rare troglodytes *Caecobarbus geertsi* and *Anoptichthys jordani* expel their eggs in a more or less haphazard fashion, the substrate on which they spawn must have crevices and pores into which the adhesive eggs can roll and be hidden so they will not be eaten by their parents or other cave inhabitants. No parental care has been observed and the fish presumably follow the reproductive habits of their predecessors (*Astyanax mexicanus*) which live above ground (Cahn 1958). Nonetheless, the specialized morphophysiological adaptations of the adults are paralleled by characteristic adaptations in egg number and size, and in length of embryonic development. Only a few large eggs are produced and the embryonic period is extended, much as in the preceding guild. Well-developed larvae or juveniles probably emerge from these large eggs. The large size of larvae and ability to feed on larger items guarantees good survival for those few needed to keep the population at a constant density (Mohr and Poulson 1966). The large yolk can also serve as a surface for expanded embryonic respiratory organs (Fig. 6c) which are so essential in the hypoxic cracks where the eggs develop, and when fanning action of the guarding parents is not available. Nothing, however, is as yet known about the spawning habits of these fishes in nature (Lüling 1954).

Provisionally another species can be included here. According to Hayley (1957) captive *Rasbora trilineata* spawns only in darkened aquariums and deposits adhesive eggs under an object on the bottom. However, this has not yet been confirmed by other authors, and variation in egg number and size of eggs is unknown. It is likely that the South African *Clarias cavernicola* Trewavas, 1936 belongs in this guild, but nothing is known of its breeding habits and development (Jubb 1967). Probably other fishes belong to this guild and will be discovered later (Riedl 1966). *Stygichthys typhlops* is such a recent discovery.

There is no representative of this guild within the freshwater fishes of Canada and Europe.

Ostracophils (A.2.3) have already been well defined by Kryzhanovsky (1949), although he considered them a homogenous group of the subfamily Acheilognathinae (Rhodeinae) only. We now know that a similar strategy is used by some Gobioninae, liparids, and at least one aulorhynchid.

Fishes of this guild hide their eggs in the gill chambers of live mussels (*Unio*, *Anodonta*, *Cristaria*, etc.), crabs (*Paralithodes camtschatica*, *Lopholithodes foraminatus*), or ascidians (*Cynthia roretzi*). Species of different phyletic lines have developed this ability and represent a remarkable case of convergence. All females of this guild grow an ovipositor used to introduce eggs through the excurrent or incurrent siphon, or through the gill apertures of the host. As a rule it is the males that select the host. They eject their sperm before or after the eggs have been deposited (Wickler 1973, p. 34–35). The sperm is inhaled by the host which facilitates fertilization of the eggs.

Fecundity of members of this guild is low since the host, in which the eggs are hidden and incubated, protects them efficiently from predators. However, one host can harbor some 100 or more eggs deposited by different females (Balon 1962a, 1967a, Photograph 91) or even by one female only (Peden and Corbett 1973). Furthermore, there is a higher proportion of yolk in their eggs, relative to vegetative plasma, than in other fishes. The yolk is also more concentrated, which prolongs the time of embryonic development and enables the larva to leave the host in a well-advanced state. Special adaptations were necessary to enable incubation in such a strange environment (inside another animal). Adaptation to a possibly low oxygen content probably causes an extremely slow pace in the early stages of development. This adaptation is irreversible, because eggs extracted from the mussel and incubated in clean, well-oxygenated water develop no faster than those within the host's body, and most of them die.

Very strong embryonic respiratory organs develop, and a dense net of capillaries from the subintestinal vein forms on the surface of the yolk. Long, numerous loops of segmental vessels and of *vena caudalis inferior* form dense respiratory areas in the dorsal and ventral postanal fin fold (Fig. 7). These temporary respiratory organs are retained until the larva leaves its host.

In some species special temporary structures develop to oppose the possible premature expulsion from the host (Fig. 7a, b). Eggs of acanthorodeinids, which are reported to be more numerous but smaller than eggs of rhodeinids, hatch very early and become covered with spine-like epidermal scales (Fig. 7c). The premature eleutheroembryos are capable of moving in a worm-like manner and crawl away from the initial, closely packed group in an attempt to reach better oxygen conditions. Eggs of rhodeinids, which number less than 100 in one female, are larger than those of acanthorodeinids and are deposited individually within the gill lamellae of the host; the eleutheroembryos cannot crawl and develop no spiny scales but the anterior part of their yolk forms a pair of folds, bordered by glands of an unknown function (Fig. 7b). These folds may fix the eleutheroembryo inside the mussel. However, as soon as the first pigment appears in the eyes, all embryos of this guild become highly photophobic, which presumably prevents a premature departure.

Eggs of liparids are deposited in a densely packed group in one of the gill cavities of the king or box crab. The mass of eggs causes a collapse of the host's gills (Peden and Corbett 1973, Fig. 1 and 2). While the yolk is still very large, the embryos have fully differentiated fins (Fig. 6d) and are, in this respect, similar to other members of this guild.

Cyprinid fishes, like *Rhodeus sericeus*, *R. ocellatus*, *R. atremius*, *Acheilognathus moriokae*, *A. tabira*, *A. cyanostigma*, *A. rhombea*, *A. longipinnis*, *A. lanceolata*, *A. limbata*, *Tanakia tanago*, and *Pseudoperilampus typus* deposit their eggs in the suprabranchial cavity of mussels; *Sarcocheilichthys variegatus* in the mantle cavity or in the incurrent siphon of mussels (Nakamura 1969); the aulorhynchid *Aulichthys japonicus* in the peribranchial cavity of ascidians; and the liparids *Careproctus* spp. in the gill chamber of the king and box crabs (Rass 1950; Vinogradov 1950; Hunter 1969; Parrish 1972; Peden and Corbett 1973). Kryzhanovsky et al. (1951) indicated that *Sarcocheilichthys nigripinnis czerskii* (syn. *Chilogobio soldatovi*) may be an ostracophil, but Banarescu and Nalbant (1973) and Banarescu (1974) concluded that females of all species and subspecies of the East Asian cyprinid genus *Sarcocheilichthys*, except *S. sinensis* (Fig. 1b), have long ovipositors and "lay the eggs in the branchial cavity of living fresh-water mussels." However, there is neither convincing evidence of this available nor any data on their development.

A species from Lake Tanganyika may also belong to the same guild. Vanderplank (1941) found eggs in the mussel of the genus *Spatha* in the resting phase during the dry season. A similar phenomenon was reported recently by Able and Castagna (1974), who found *Fundulus heteroclitus* eggs in the shells of *Modiolus demissus*, exposed to desiccation at low tides in Virginia. The latter would be the first such case reported from the North American continent. Both the Virginia and Tanganyika reports have to be investigated further; in the end it may be found that the two species of fish are closer to one of the following two guilds than to ostracophils.

No Canadian species belong to this guild. *Rhodeus sericeus amarus* is the only European representative.

Aero-psammophils (A.2.4) bury their eggs out of water in the sands of sea beaches. Their eggs are incubated in the damp sand until the returning high tide washes them back to sea. The timing of the spawning act is surprisingly perfect. It occurs only during a single hour or so during consecutive days of a single week and at extremely high tide followed 14 days later by another slightly higher tide.

Only three unrelated species of this guild have been studied in detail. *Leuresthes tenuis* (night spawner) and *L. sardina* (day-time spawner) have pink, nonadhesive, hard eggs 2 mm in diameter. When the female reaches the highest point of the surf with one of the farthest running waves

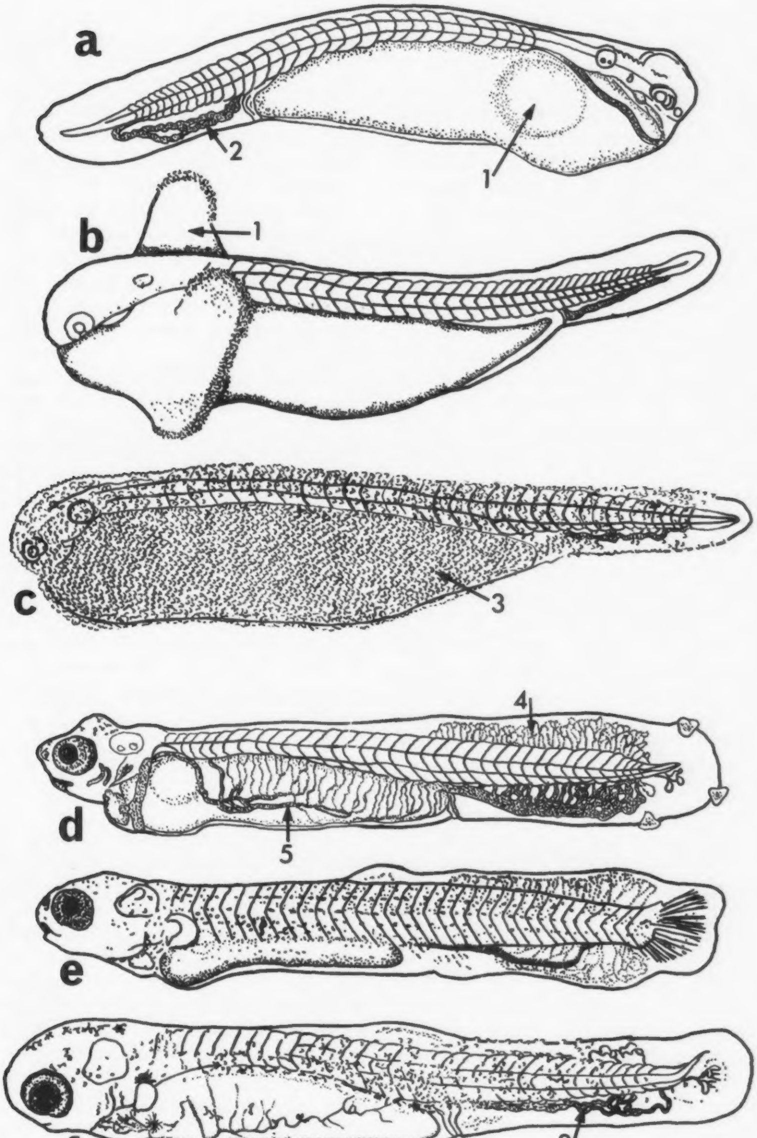

FIG. 7. a, Embryo of *Rhodeus sericeus amarus* 6 mm long; b, *R. sericeus sericeus* 5 mm long, and c, *Acanthorhodeus asmussi* 3 mm long (1 = folds of yolk, 2 = lower caudal vein); d, older embryo of *R. s. amarus* 8 mm long; e, *R. s. sericeus* 8 mm long and f, *A. asmussi* 6 mm long (4 = segmental respiratory vessels in dorsal fin fold, 5 = respiratory extension of subintestinal vein on the yolk). After Kryzhanovsky 1949 and Kryzhanovsky et al. 1951.

and if there are males close at hand, she buries herself out of water, tail first, in the sand. The males release sperm in the sand next to the female while she deposits her eggs. After approximately 2 weeks a new high wave with multiple receding wavelets uncovers the embryos (Fig. 8d) which hatch as they are freed. The Japanese fugu puffer, *Spheroides niphobles*, spawns in much the same way but the eggs are deposited not so far out of the water and measure only 1 mm in diameter. They hatch in less than 1 week (Uno 1955) only if appropriate rolling motion triggers the hatching. In both cases the ability of eggs to develop in damp air was reported independently; in water and under different temperatures no changes in the rate of development were demonstrated, which led to the conclusion that the peculiar and elaborate spawning habit "serves merely to give protection to the egg" (David 1939).

Xerophils (A.2.5) are a specialized guild of fishes with a short life span adapted to the seasonal desiccation of their habitat. These fishes inhabit, almost exclusively, small intermittent water bodies, i.e. isolated pools and swamps in the African savannah or the South American llanos and pampas that dry up completely during the dry season. Eggs are deposited in the bottom sod, sand, or most frequently, mud (e.g. Wickler 1973, p. 106) and they survive the several months of dry season under the cracked dry crust of the damp sod or mud. In extremely dry years the embryos of xerophils can survive as long as 18 months and more in the egg membranes lodged in the dry mud. Eggs of *Cynolebias elongatus* survived this period in dry peat under laboratory conditions and I was able later to induce hatching of viable fish. When the pools refill after the first rains, the embryos hatch and grow so rapidly that within several weeks they are able to reproduce. When the water begins to recede at the beginning of a new dry season, a large number of eggs have already been deposited along the subsequently exposed shore line and egg laying continues almost until the pool dries up and all adult fishes die. Myers (1952) characterized these species as "annuals." The whole population survives only in the egg membranes, as hibernating embryos. The embryonic period lasts nearly as long as the remaining larval, juvenile, adult, and senescent periods together.

In members of this guild the adaptations for survival rest mainly with some unique embryonic characteristics. The eggs can arrest their development at three different times. The first resting time (diapause I), usually facultative, occurs early in the cleavage phase (Fig. 8a). The cells disperse and rest for several weeks. Later embryogenesis commences from their reaggregated mass. The two other resting periods (diapause II and III) are usually obligate (Fig. 8b, c). During the time of developmental arrest, yolk reserves remain intact and blood circulation stops entirely. Heart contractions are occasional and feeble (Peters 1963, 1965; Wourms 1967, 1972a, b, c). Oxygen presumably reaches the eggs through pores in the dry soil and is considered the main regulating factor for the resting periods. Hatching is induced by reduction of oxygen occurring when rain water clogs the pores in the mud, and by the pressure of the water column above the eggs (Morenski 1974).

To date, xerophils are known only from cyprinodontid fishes of Africa and South America. The following "have been shown to have a dispersion–reaggregation pattern of development and the ability of diapause" (Wourms 1967): *Aphyosemion arnoldi*, *A. caeruleum*, *A. nigerianum*, *A. seymouri*, *A. walkeri* (names not updated), *Austrofundulus myersi*, *A. transilis*, *A. dolichopterus*, *Cynolebias bellotti*, *C. nigripinnis*, *C. whitei*, *C. melanotaenia*, *C. wolterstorffi*, *Nothobranchius guentheri*, *N. melanospilus*, *N. orthonotus*, *N. palmquisti*, *N. rachovi*, *N. taeniopygus*, *N. furzeri*, *N. kirki*, *Pterolebias longipinnis*, *P. peruensis*, *P. zonatus*, *P. maculipinnis*, *Rachovia brevis*, *R. hummelincki*, etc.

B. Guarders

SUBSTRATUM CHOOSERS (B.1)

Guarding is a solely ethological aspect of guilds. It has, however, profound ecomorphological consequences. Better protected from enemies, guarded eggs need not be numerous to assure survival of the species. Consequently, eggs can often be larger and result in more viable offspring with less food specialization. (Incidentally, external food independence is known to be assured by other means also.) Furthermore, spawning sites with low oxygen content can be used because the guarding parents clean the eggs and produce a flow of water around them by fin-fanning and oral ventilation. "In all known fishes that are parental, outside of the Cichlidae, the male is the parent. The evolutionary progression has been from (i) an exclusively parental male, to (ii) shared parental care by the male and female, to (iii) a division of roles with the female as the direct parent and the male as guardian, to (iv) polygyny (Barlow 1964). This is clearly a forward progression resulting in a more efficient division of labor between the male and female" (Barlow 1974).

Fishes that do not build the complicated struc-

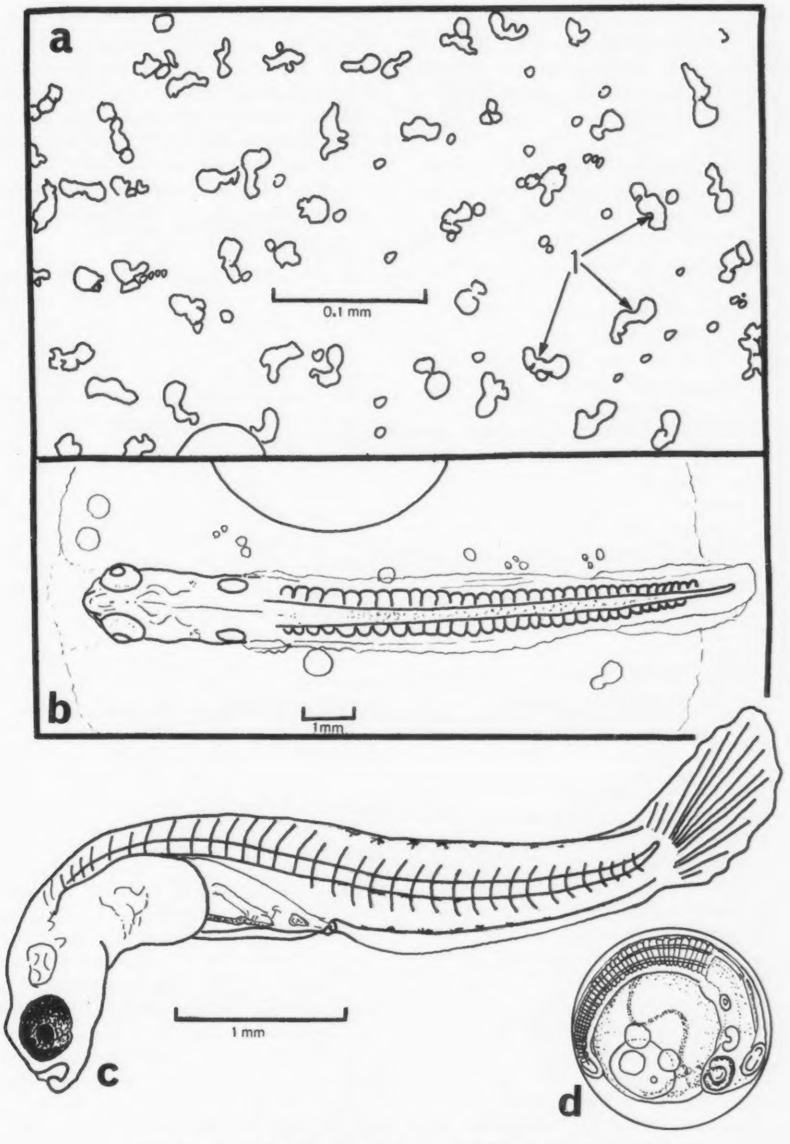

FIG. 8. a–c, Resting times in annual fishes: a, diapause I in dispersed cell phase of *Rachovia brevis* (1 = dispersed cells), b, diapause II during the long somite embryo phase of *Austrofundulus myersi*, and c, diapause III in *Pterolebias longipinnis* just before hatching, 6 mo after fertilization; d, Embryo of the grunion *Leuresthes tenuis* 60 h after fertilization. After (a, b) Wourms 1972c, (c) own unpublished material, and (d) David 1939.

tures, subsequently called nests, but that deposit their eggs on top of a selected object, are included in this subsection. The surface chosen for egg attachment may be cleaned and guarded in advance of the spawning act.

Lithophils (B.1.1) choose rocks for attachment of their eggs. The eggs are often more or less cylindrical and one pole is equipped with fibers that firmly adhere to the rock (Fig. 9a). Males of *Loricaria parva* and *L. macrops*, however, sit on their eggs like hens, cleaning them with a mouth-sucking motion and fanning them with a swinging motion of their whole body. Their eggs are spherical and have an adhesive membrane but no fibers. *Chromis chromis* of the Mediterranean deposits its eggs on slanting rock surfaces in spawning colonies, and the male fans them with his tail (Abel 1961). *Cyclopterus lumpus*, which can also be fitted into this guild, deposits a massive, spongy cluster of pink eggs over 20 cm in diameter. The female attaches them to rocks and until they hatch 2 months later, the male fans and ventilates them with jets of water from his mouth (Mochek 1973). *Tetraodon cutcutia* and *T. fluviatilis* are reported to do the same, but the eggs hatch in a week.

Europe: *Gobius melanostomus*, *G. gymnotrachelus*, *G. batrachocephalus*, *G. cephalarges*, *G. kessleri*, *G. ratan*, *G. syrman*, *Benthophilus stellatus*.

Phytophils (B.1.2) in this subsection scatter their eggs or attach them to submerged plants. The males guard and fan them. The place of egg attachment is sometimes selected and cleaned in advance of the spawning act and sometimes not. A variety of aquatic plants, with no marked preference, are used by *Polypterus*, *Paracheirodon innesi*, *Silurus glanis*, *Jordanella floridae*, *Labrus herggylta*, *L. turdus*, and *Sardelia capensis*. *Archoplites interruptus*, *Pomoxis annularis*, *Elassoma zonatum*, and *Clinus testudinarius* deposit their eggs in algal growth. Fishes of the genus *Pyrrhulina* prefer broadleaved plants and *Leucaspius* attach ribbon-like threads of eggs to broad leaves or around stems. The male of *Polypterus* also guards the larvae and in *Sardelia capensis* both sexes are reported to guard the eggs.

No cement glands are known in the members of this guild. So that the eleutheroembryos avoid contact with the hypoxic muddy bottom, the embryonic period is prolonged and the newly hatched larvae swim instantly. Probably as a result of the parents' fanning activity (Mertz and Barlow 1966) the embryonic respiratory organs are feeble, a characteristic of nonguarded lithophils (A.1.3) (Fig. 9e, f, g) also. Guarded phytophils developed this characteristic possibly as an adaptation to lentic conditions (Kryzhanovsky 1949). In other members of this guild with bottom-dwelling larvae (Fig. 9c, d) the development of external gills and large pectorals (Kerr 1907) compensates for the lack of oxygen.

Canada: *Pomoxis annularis*.

Europe: *Umbra krameri*, *Leucaspius delineatus*, *L. marathonicus*, *L. stymphalicus*, *Silurus glanis*, *S. aristotelis*.

Aerophils (B.1.3). So far as is known, this unique adaptation is exhibited by a single species, *Copeina arnoldi*. The eggs are not deposited in water but on undersides of broad leaves or rocks that overhang the water. The eggs are attached during the spawning act, when female and male leap out of the water together and cling upside-down to the chosen substrate, usually about 10 cm above the surface of the water. Every 20 minutes or so the male splashes the eggs by swishing his tail, using the edge of the egg attachment substrate as an aiming cue (Krekorian and Dunham 1972). Three days later the embryos hatch and drop into the water. Like phytophils, the embryos have cement glands and attach themselves for a short time to plants near the surface of the water. As a result of good oxygen conditions embryonic respiratory vessels are probably not well developed. Mating out of water occurs in *Aphyocharax rubripinnis* (Innes 1935) and in some *Glandulocaudines*. Aerophilous reproduction could have developed from this aerial mating which initially facilitated fertilization only (Nelson 1964). Protection from enemies seems to be the reason for this adaptation.

Pelagophils (B.1.4) in this subsection are a specialized guild, which includes to my knowledge, only *Ophiocephalus* spp. and *Anabas* spp. (Barlow et al. 1968). Their eggs are nonadhesive and positively buoyant. Deposited on the surface of the water in openings between vegetation growing in the shallows, they float in a cluster; and propelled by wind, they often drift into remote places. Both the male and female guard the eggs and larvae. Judging from data on development (Soin 1960) and from the need of the adults for complementary aerial respiratory organs, having pelagic eggs seems to be an adaptation to hypoxic conditions. As these pelagic eggs are, at the same time, vulnerable to predation, guarding evolved to ensure protection against enemies living in such habitats.

NEST SPAWNERS (B.2)

Some members of this subsection build more and some less elaborate structures for egg deposition or for guarding the hatched offspring, whereas

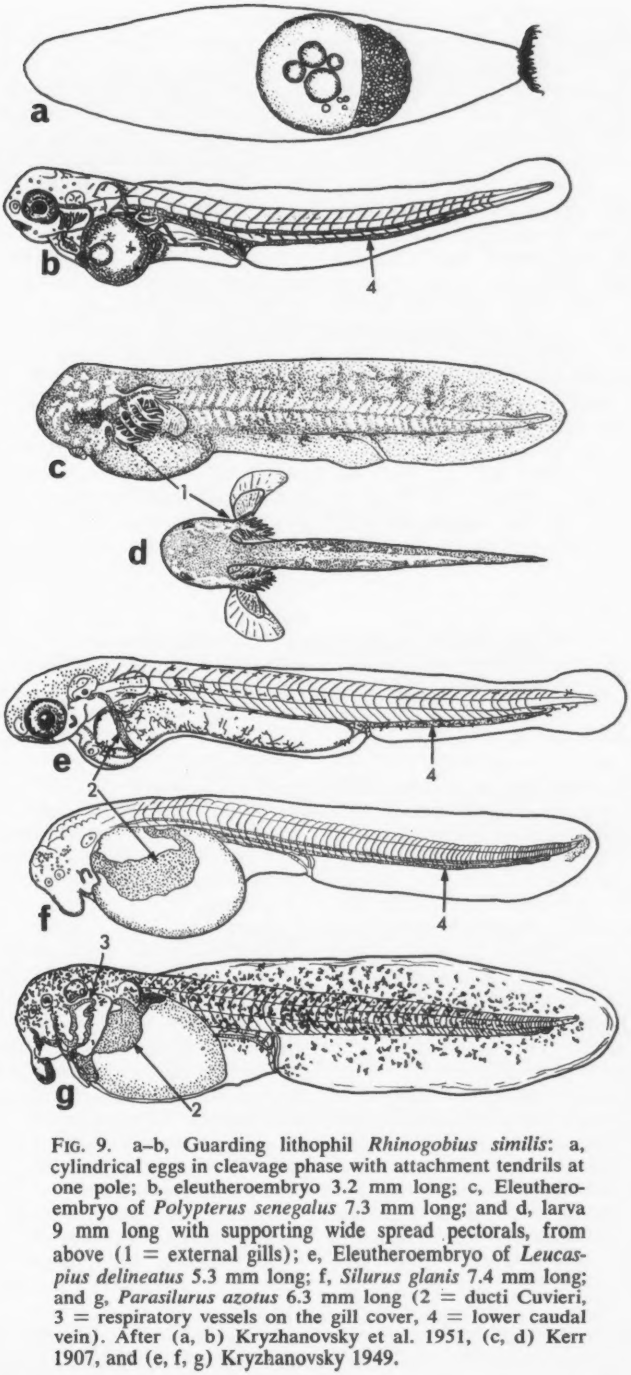

FIG. 9. a–b, Guarding lithophil *Rhinogobius similis*: a, cylindrical eggs in cleavage phase with attachment tendrils at one pole; b, eleutheroembryo 3.2 mm long; c, Eleutheroembryo of *Polypterus senegalus* 7.3 mm long; and d, larva 9 mm long with supporting wide spread pectorals, from above (1 = external gills); e, Eleutheroembryo of *Leucaspius delineatus* 5.3 mm long; f, *Silurus glanis* 7.4 mm long; and g, *Parasilurus azotus* 6.3 mm long (2 = ducti Cuvieri, 3 = respiratory vessels on the gill cover, 4 = lower caudal vein). After (a, b) Kryzhanovsky et al. 1951, (c, d) Kerr 1907, and (e, f, g) Kryzhanovsky 1949.

some only clean the place of the future egg attachment. Some clean by sandblasting, whereas others are less thorough. Eggs are guarded though this is not always the case with the hatched offspring (Mochek 1972). But if the offspring are guarded they are also herded, e.g. into bottom pits, for some time.

Lithophils (B.2.1) represents a polyphyletic guild and their eggs are deposited in single-layer or multilayer clutches on cleaned areas of rocks or in pits dug in gravel. Numerous deviations from this simple scheme have been recorded in the mode of nest construction and parental care. For example, males usually guard only the eggs deposited in gravel nests built in shallow running waters. The nest of *Myoxocephalus quadricornis* is built as a large pit in a soft mineral bottom or on a flat circular clearing amid algae and the male guards the egg cluster for an extended period of time (Westin 1970). The nest of *Leporinus affinis* is formed as a cavity in sand paved with pebbles. *Notropis cornutus* sometimes builds its own nest in the gravel but prefers to occupy, in cuckoo–cowbird fashion, those of *Hybopsis micropogon*, *H. biguttatus*, *Semotilus corporalis*, *S. atromaculatus*, *Exoglossum maxillingua*, *Campostoma anomalus*, or *Micropterus dolomieui* if available. A number of males may eventually spawn over one nest. In *Hexanematichthys australis*, eggs are buried under a mound of gravel. *Dormitator maculatus*, *Mogurnda mogurnda*, and *Crassiops gali* attach their eggs to cleaned rock surfaces. All these nests are guarded.

In cichlids parental care is usually extended and includes care of the offspring after hatching. Frequently both parents guard, but "the female does most of the direct parental care while the male guards the territory or remains nearby until the fry begin swimming. Then both sexes guard them. There is evidence of an inclination toward polygyny, but this is counteracted by selective pressure from predators, requiring both parents for protection of the fry" (Barlow 1974).

Eggs are attached to cleaned places on rocks, and one or more pits are dug on the bottom, to which the offspring are transferred by mouth after hatching. At nightfall, the swimming larvae and juveniles are often herded into these pits. This applies to *Aequidens pulcher*, *Astronotus ocellatus*, *Cichla ocellaris*, *Cichlasoma facetum*, *C. severum*, *C. nigrofasciatum*, *C. cyanoguttatum*, *C. biocellatum*, *Pelmatochromis arnoldi*, and many others. The females of *Cichlasoma aureum*, *C. tetracanthum*, *C. cutteri*, *C. haitiensis*, and *C. meeki*, do most of the egg incubating and the males most of the pit digging. The eleutheroembryos of *C. bimaculatum* and *C. festivum* are not placed in the pits but are hung on plants. In *Heterotilapia multispinosa*, plants or vertical surfaces of submerged objects are used if mud and detritus make the use of bottom pits impractical (Baylis 1974).

"Not only do the parents defend the fry, but in many species they also help provide food. In some species the parents turn over leaves for the offspring ... When the fry are extremely hungry in almost any Central American species they will begin to graze on the mucus on the sides of their parents (*C. nigrofasciatum*, *C. spilurum*, *C. macracanthum*, *C. friedrichstahlii*, *C. beani*, *C. longimanus*). In *C. citrinellum* the response is apparent even when the fry are only modestly hungry (Noakes and Barlow 1973), as is also true of *C. labiatum*" (Barlow 1974). *Geophagus brasiliensis* picks up its offspring by mouth more readily and after less provocation than most cichlids, whereas the eggs of *G. jurupari* and *G. balzani* are actually guarded by both parents in the nest for 1 day only; then the female collects them into her mouth and broods them in a regular bearers' (C.1.3) manner. This mouth-bearing phenomenon occurs several times in different guilds of this subsection and clearly indicates the polyphyletic evolution of mouth-brooding habits (Breder 1933).

The eggs are always spherical or elliptical and stick firmly to the substrate. Size varies greatly, as does color. *Pylodictis olivaris* eggs are deposited in a thick adhesive mass that "shake[s] like a bowl of jelly" when the male fans them, whereas individual eggs of *Pelmatotochromis subocellatus* and *Mogurnda mogurnda* are attached to the substrate by short stalks that wave under the influence of fanning.

In some members of this guild the photophobic reaction of A.1.3. lithophils is retained and the eleutheroembryos hide under the gravel of their nests and are guarded. But in the majority, the need to hide is replaced by parental care. Some that build their nests in running water have moderately developed embryonic respiratory organs; others from lentic nest sites have highly developed yolk and fin fold vessels (Fig. 10a). Most eleutheroembryos have cement glands (head glands) and swing their tails rapidly while attached to the substrate.

Canada: *Notropis cornutus*, *Exoglossum maxillingua*, *Ictalurus melas*, *Ambloplites rupestris*, *Lepomis auritus*, *L. cyanellus*, *L. macrochirus*, *L. megalotis*, *Micropterus dolomieui*, and *Myoxocephalus quadricornis*.

Europe: *Benthophiloides brauneri*, *Myoxocephalus quadricornis*.

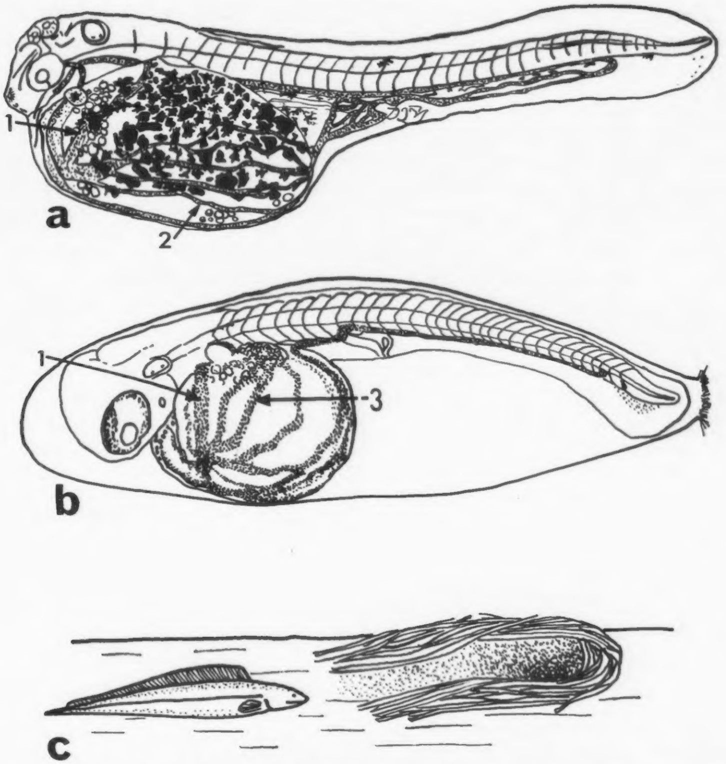

FIG. 10. a, Eleutheroembryo of *Cichlasoma cyanoguttatum* 4.2 mm long (1 = ducti Cuvieri, 2 = respiratory branches of subintestinal vein); b, embryo of *Percottus glehni* 3.7 mm long (3 = respiratory branches of hepatic vein); c, floating nest of *Gymnarchus niloticus*. After (a) Balon 1959b, (b) Kryzhanovsky et al. 1951, and (c) Daget 1952.

Phytophils (B.2.2) in this subsection are a polyphyletic guild very much like the preceding one. Its members, however, are adapted to nesting above or on a soft muddy bottom. For example, *Amia calva*, *Clarias batrachus*, *Enneacanthus gloriosus*, *Pomoxis nigromaculatus*, *Elassoma zonatum*, *E. evergladei*, *Etheostoma blennioides* and *Stizostedion lucioperca*, *S. marinum*, and *S. volgense* construct circular nests on the bottom amid algae and vascular plants whose roots are often exposed (Dmitriyeva 1973); nests are built and eggs are guarded by males. *Etheostoma microperca* and *Perccottus glehni* most often attach their elliptical eggs to stems of plants, whereas the female of *Heterostichus rostratus* constructs a nest in the kelp and lays eggs that are bound together with adhesive threads and defended by the male. *Pterophyllum scalare* and *P. eimekei* attach their eggs to a thoroughly cleaned surface on a broad leaf and immediately transfer the newly hatched eleutheroembryos to another cleaned leaf. Several cleaned "nursery areas" on a number of leaves are maintained simultaneously. If the young get detached and fall while swinging their tails too vigorously during respiratory movements, each is picked up and returned in their parents' mouths to the plants where they hang by means of cement glands. The same behavior is reported for *Nannacara taenia*, but in addition, the guarding parents can signal danger by quivering their fins and bodies. At this the young hide on the bottom or remain motionless close to their parents. *Amblydoras hancocki* builds a nest of detached leaves, in which a flat cluster of eggs is covered by more leaves and guarded by both parents. "*Aequidens coeruleopunctatus* breeds in streams in Panama where there is little hard substrate on which they can deposit their eggs. The female selects a relatively rare rubbery leaf from among the litter on the bottom and carries it past the male during courtship. Later she spawns on the leaf. ... When danger threatens she grasps the leaf in her mouth and pulls it back under the bank of the stream or into shallow water" (Barlow 1974).

Perccottus glehni attaches cylindrical eggs to stems by threads on one pole (Fig. 10b). A more refined type of attachment occurs in *Monocirrhus polyacanthus*. "The eggs when first laid are close to the leaf to which they are attached, and the supporting threads are tightly coiled into a fine spiral. As development proceeds, this sags down so that, by the time of hatching, the eggs hang farther from the leaf on a straight thread."

The male of *Gymnarchus niloticus* builds a floating nest of plants approximately 90 × 65 cm (Fig. 10c) in dense swamps. Amber-colored, spherical eggs, 10 mm in diameter (sic) hatch in 5 days and after 18 days the larvae are ready to leave the nest. The guarding male is very vicious. *Polycentropsis abbreviata* attaches its eggs to the underside of cleared areas on a broad leaf, but in addition the male surrounds them with bubbles of air fetched from the surface. A bottom pit is then prepared under this leaf into which the hatched eleutheroembryos drop. They soon leave this pit to hang briefly on plants. *Osphronemus goramy* builds its nest of plant fibers in midwater, and incorporates some mucous air bubbles in the construction. The eggs and young are positively buoyant and have to be kept under the nest canopy by the guarding parents. The froth nest builders (B.2.4) may have evolved from here.

Canada: *Amia calva, Micropterus salmoides, Pomoxis nigromaculatus.*

Europe: *Gobius ophiocephalus, Stizostedion lucioperca, S. marinum,* and *S. volgense.*

Psammophils (B.2.3) in this section form a guild for which relevant data are available for only a single noncichlid fish and one cichlid fish. *Abbottina rivularis* males "build special nests on the bottom; of [sic] shallow water (no deeper than 40 cm, at about 1 m from the shore); the nests are circular, their upper diameter is 12–43 mm, their depth 5 mm. [Nest photograph in Nakamura 1963, fig. 47c.] A female spawns several times in a season. The males guard the nest. The diameter of the egg is about 2–2.5 mm. The larvae hatch out in 6–8 days (at 18°C); they lay [sic] usually on the bottom and move very little" (Banarescu and Nalbant 1973). The eggs have a thick secondary membrane and cover the entire bottom of the nest in a layer over 2 cm thick (Nakamura 1969). At first, each egg is completely covered with sand grains, but during incubation the egg membrane loses its adhesiveness and the sand is gradually washed off. After hatching, the eleutheroembryos and larvae remain in the nest and maintain a standing position while leaning on their wide pectorals (Fig. 11a–c). Embryonic respiratory organs are feebly developed (Kryzhanovsky et al. 1951). *Abbottina rivularis,* like the A.1.6 psammophils, has long *cupulae* at the beginning of exogenous feeding (see possible significance in Balon 1960).

Cichlasoma nicaraguense "digs a remarkably deep hole in the sand by a rock ... The eggs are not only large but rather buoyant, bouncing around in the bottom of the nest. The absence of adhesive threads, large size, and buoyancy are adaptations to prevent loss of the eggs in the sand. While the female does some fanning of the eggs, she also does an inordinate amount of taking the eggs into her mouth and spitting them out (Stratton, 1968). Apparently this species is but one step away from becoming a mouthbreeder" (Barlow 1974).

Aphrophils (B.2.4). A unique habit among characids was reported for *Hepsetus odoe.* This common African pike-like fish builds nests of floating froth, 5 cm deep and about 20 cm in diameter, usually placed in grass or reeds along the shores. The eggs, 2.5 mm in diameter, are mixed with the froth and after hatching, the eleutheroembryos leave the froth to hang from the water surface by their cement gland. The froth nest is built by both parents and is maintained and guarded by one or the other, or by both (Svensson 1933; Jubb 1967).

In *Callichthys callichthys* a floating nest of froth "is constructed by the male who takes the air into his subterminal mouth in an inverted position and emits mucus-covered bubbles from his gill cleft." Only the male maintains and guards the nest. The froth nest of *Haplosternum* sp. is placed under a floating raft constructed of aquatic plants. The female practices an oral transfer of sperm, and deposition of egg groups "cupped between her ventral fins."

Among anabantids a surface froth nest is built by *Macropodus opercularis, Trichogaster trichopterus, T. leeri, Trichopodus pectoralis, Parasphromenus deissneri, Colisa labiosa, C. lalia* and *Betta splendens.* The floating or sinking eggs (presumably according to the different structure of the eggs, Soin et al. 1973) are picked up, blown

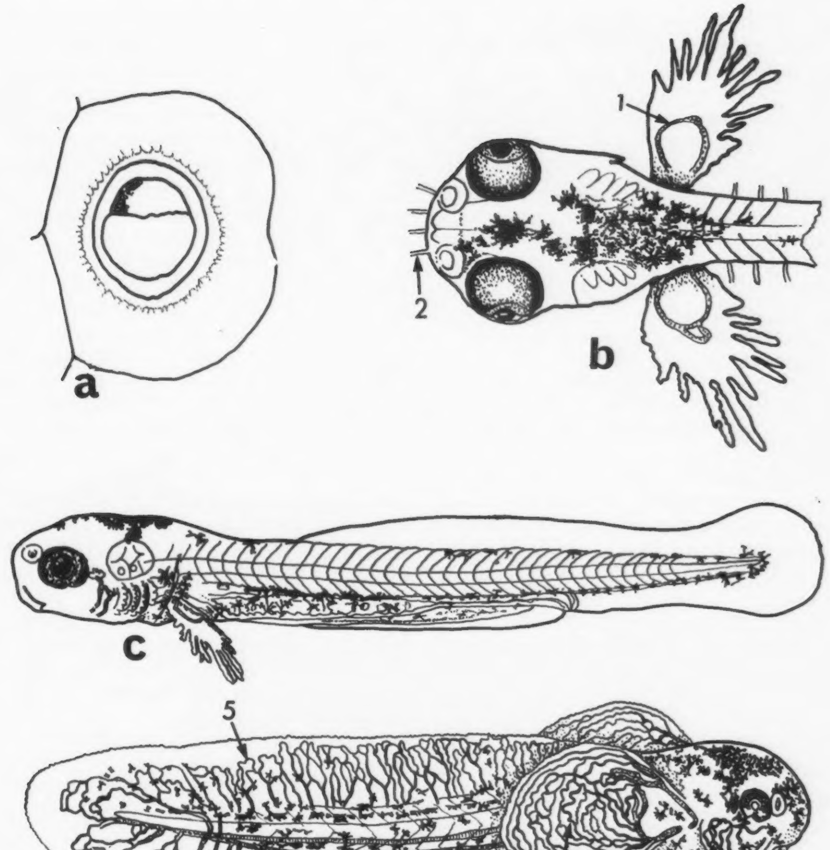

FIG. 11. a–c. Psammophilous nest spawner *Abbotina rivularis*: a, egg in gelatinous secondary membrane, b, protopterygiolarva 5.1 mm long from above (1 = respiratory snares of vessels in pectorals, 2 = cupula), c, same, from the left side; d, eleutheroembryo of *Haplosternum* sp. 6.5 mm long (3 = respiratory vessels in gill cover, 4 = respiratory vessels in pectorals, 5 = respiratory vessels of segmental and caudal veins in embryonic fin fold); e, anterior part of *Macropodus* sp. with respiratory vessels (see Fig. 10b for explanations). After (a) Nakamura 1969, (b, c) Kryzhanovsky et al. 1951, (e) 1953, and (d) Kryzhanovsky 1949.

into the bubble nest, and cared for by the male. *Sphaerichthys osphromenoides* and *Betta brederi* have been reported to build froth nests while keeping some of the eggs in the mouth or sometimes even practicing mouthbreeding (Kühme 1962). Sometimes the female blows a few mucous bubbles and cares for some of the eggs; more often, however, she is driven away by the male. *Macropodus cupanus* builds a less substantial bubble nest that is often in midwater among plants, or even in a shell on the bottom. *Colisa fasciata* reinforces its froth nest with small plant fragments. The floating eggs of *Belontia signata* and *Ctenops vittatus* are placed in a few bubbles only.

"The froth nests of these fishes are considered of importance as a depot for the eggs, protection from suffocation, protection from sunlight, attractant for microorganisms on which the fry can feed, an oxygen reservoir for the developing fry, and as an anchor for the eggs amid plants. To this list might be added a protective effect against small marauders, such as other small fishes and aquatic insects." The embryonic respiratory organs on the yolk are known to be well developed (Kryzhanovsky et al. 1953) in spite of good oxygen conditions within the froth nest (Fig. 11d, e).

Speleophils (B.2.5) are a large polyphyletic guild of fishes guarding their spawn in natural holes and cavities or in specially constructed burrows. The majority simply deposit their eggs on a cleaned area of the undersurface of flat stones and the male guards the eggs. The eggs can be in a single layer or in two, as well as in a multilayered mass. The eggs will, in general, have good oxygen conditions during their incubation (Morris 1954), and thus, the embryonic respiratory organs are only moderately developed (Fig. 12a). Many fishes can be fitted in this guild, as for example, *Pimephales notatus*, *Noturus flavus*, *Astroblepus chatae*, *Therapon jarbua*, some *Etheostoma* spp., some *Gobius* spp., *Sicydium japonicum*, *Ernogramus hexagrammus*, *Ophidion elongatus*, *Artedius fenestralis*, and *Cottus* spp.

Eupomacentrus leucorus builds its nest in similar holes under rocks; however, the cavity is first thoroughly sandblasted. Egg attachment on a vertical rock surface was reported in captive fish (Breder and Coates 1933), and this indicates the possible origin of the strategy to guard spawn in natural cavities which give more protection than an exposed substrate. The related *E. leucostictus*, as well as *Abudefduf zonatus* and many other members of this guild guard their spawn inside empty shells or under stones or shells (Keenleyside 1972). In some, the eggs are spherical, in others, more or less elliptical or cylindrical (Breder 1943); some of them have special adhesive threads at the pole of attachment. Others are glued together by a sort of secretion (*Ancistrus anisitsi*). In the last mentioned example strong embryonic respiratory adaptations to hypoxic conditions are present.

In *Anoplarchus purpurescens* and *Macrozoarces americanus* the female guards the adhesive eggs under stones and often she can be found coiled around them. *Eleotris oxycephala* has pear-shaped eggs with a short stem that attaches to the inner surface of an old piece of bamboo. The male of *Opsanus tau* guards large adhesive eggs 5 mm in diameter in a nesting cavity, for which human artifacts are preferred if available. The eggs are glued to an adhesive disc, produced before they are attached. Even after hatching, the eleutheroembryo can free itself only when the yolk is nearly absorbed, because the yolk is attached to the inner side of the egg membrane. *Paraclinus marmoratus* uses the inside of the sponge *Verongia fistularis* as spawning cavities and a few other fishes are reported to do the same. *Chaenogobius castanea* "deposits its eggs in the nearly vertical burrows of the shrimp *Upogebia*."

Other members of this guild build more or less elaborate burrows of their own. *Steatocranus casuarius* dig holes under stones; others dig them in sand or clay (*Cichlasoma maculicauda*) and may use an empty shell as a roof (*Gobius niger*, *G. minutus*). *Ancistrus anisitsi* digs tunnels in banks at the edge of a swamp, as does *Symbranchus marmoratus*. *Fluta alba* builds a U-shaped hole in a muddy bottom and is reported to have strongly developed embryonic and larval pectoral fins and fin folds with respiratory blood vessels. These fins are later fully absorbed and are absent in adults. *Pseudobagrus fulvidraco* builds vertical nest-burrows 9–14 cm deep in the bottom and the male guards the eggs (Fig. 12b). Recently *Acarichthys heckeli* was discovered to excavate tunnels in the bottom (Cichocki 1974). *Acanthogobius flavimanus* excavates Y-shaped burrows. More complex burrows are built by *Protopterus* sp., the male of which digs a long tunnel and an oval-shaped chamber, sometimes with more than one entry. The eggs are deposited inside the chamber, probably by more than one female. The oxygen concentration is very low in such a nest, where there are as many as 500 larvae at one time. The young commence air breathing only after 50 days, while still returning into the nest,

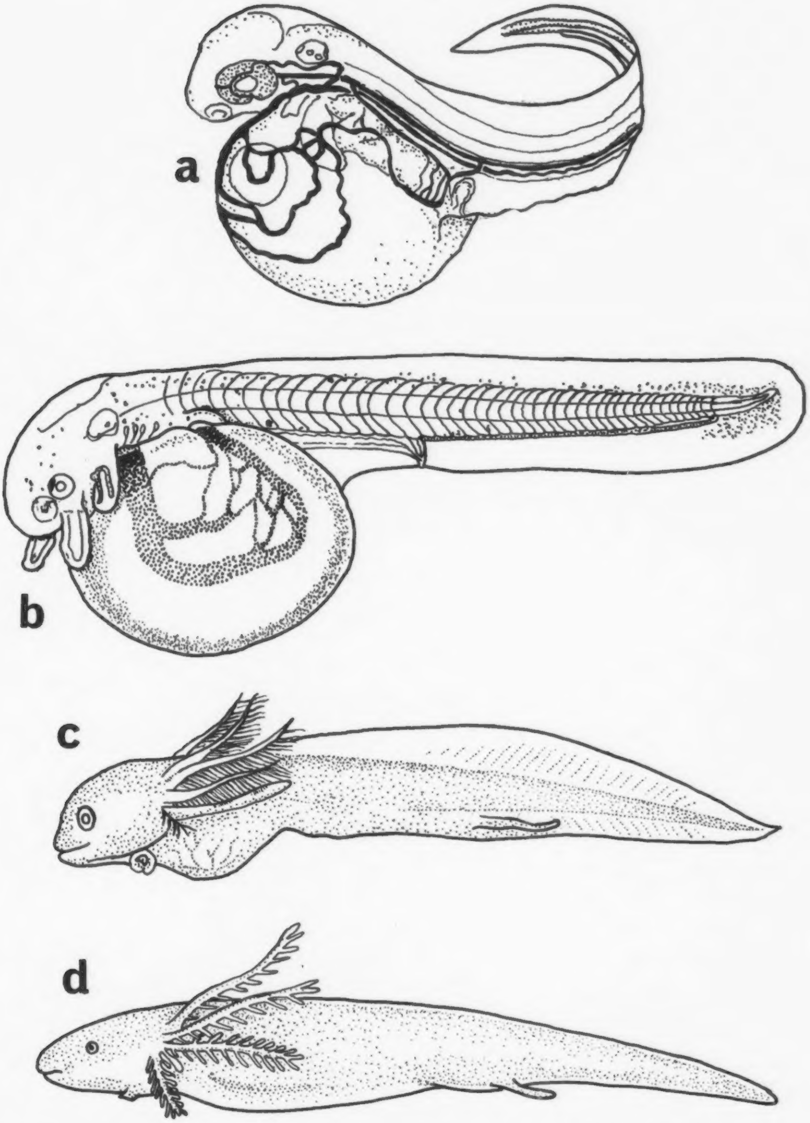

FIG. 12. a, Embryo of *Cottus* sp., b, eleutheroembryo of *Pseudobagrus fulvidraco* 5 mm long, and larvae c, of *Protopterus* and d, *Lepidosiren*. After (a) Portmann 1927, (b) Kryzhanovsky et al. 1951, and (c, d) Kerr 1910.

and when about 23 mm long (Greenwood 1958). The well-developed embryonic respiratory organs are assisted by the large external gills (Fig. 12c).

Some differences are reported in the construction of burrow-nests and in the development of eggs in different species (Brien and Bouillon 1959). Hibernation in a parchment cocoon in the dry season is in some species reported to be facultative. Adaptations for incubation under hypoxic conditions are even more elaborate in

Lepidosiren paradoxa. A long tube-like burrow with an entrance 10–12 cm in diameter is excavated on the bottom of a swamp. The burrow leads a short distance down but after an abrupt turn continues to lead for over 1 m horizontally directly to the nesting chamber. The pelvic fins of the guarding male change at nesting time into broom-like, highly vascularized organs that are able to release oxygen inside the hypoxic chamber and supply the much needed oxygen to the developing offspring (Fig. 12d).

Because of a large variety of adaptations for incubation in cavities and holes, I feel this guild is too broad to serve its purpose. Barlow's (1974) conclusion "that virtually all of the Central American cichlids are concealment breeders" expresses best the existing confusion. Fishes excavating special burrows and those guarding spawn in shallow and existing cavities, i.e. undersides of rocks and inside of shells and sponges, as well as in human artifacts (tin cans, boards, boxes) should be classified as belonging to separate guilds. However, not enough data are available on embryonic respiratory organs of either group to characterize with confidence two such separate guilds.

Canada: *Pimephales notatus, P. promelas, Ictalurus natalis, I. nebulosus, I. punctatus, Noturus flavus, N. gyrinus, N. miurus, Etheostoma flabellare, E. nigrum, Cottus aleuticus, C. asper, C. bairdi, C. cognatus, C. confusus, C. rhotheus, C. ricei.*

Europe: *Romanichthys valsanicola, Blennius fluviatilis, Padogobius nigricans, P. panizzai, Pomatoshistus canestrini, P. caucasicus, P. longecaudatus, P. microps, Proterorhinus marmoratus, Cottus gobio, C. poecilopus.*

Polyphils (B.2.6) are fishes that are not particular in the selection of nest building material and substrate. Some attach their adhesive, spherical, or cylindrical eggs to any cleaned surface (gravel, sand or sod) and subsequently guard the clutch (*Notopterus chitala, Badis badis*); others build nests of seaweeds or stones. The eggs form tough and rubbery masses that are, for example, red or yellow in *Myoxocephalus scorpius* and "maroon to bright crimson" in *Scorpaenichthys marmoratus. Hoplias malabaricus, Lepomis gibbosus, Enneacanthus chaetodon,* and *Taudanus taudanus* build circular nests (Clark and Keenleyside 1967), usually leaving sticks and rootlets in place. These nests are among or next to plants growing in muddy or sandy shallows of slow rivers or lagoons. The last species also uses its mouth to carry gravel and sticks in its nest. In all species the male guards the eggs (Fig. 13a).

Both males and females of *Arapaima gigas* build nest-holes, 60 cm in diameter and 20 mm deep, in the bottom where there is a current. The young accompany their mother, while the male guards them from some distance. White tubercles on the head of the guarding female are said to provide some kind of food for the young. In *Symphysodon discus* the nest is built or a nesting area is cleaned on rocks, sand, or plants with exposed rootlets. The mucus covering the body changes into a whitish, granular, and adhesive substance on the dorsal parts of both guarding parents and the young feed on this (Wickler 1973). In other cichlids this phenomenon occurs to a lesser degree (e.g. Noakes and Barlow 1973).

Heterotis niloticus builds a circular nest about 1 m in diameter and 20 to 60 cm deep in swamps. The rim of the nest is a high wall formed out of plant chunks. This wall is about 15–20 cm thick and projects above the water surface. The bottom is a clean platform of clay or mud. After the spawning act the fish leave by way of a hole in the wall, through which, 5 days later, the young leave the nest and are guarded by the male. For example, the nest of *Crenilabrus quinquemaculatus* "of fibrous plant material mixed with sand and gravel, was described as hemispherical in shape and exceeding 20 cm in diameter, with an opening near the bottom." The courtship and guarding of *Crenilabrus* is very similar to that of the stickleback.

Canada: *Lepomis gibbosus.*

Ariadnophils (B.2.7) are, with some exceptions, a monophyletic guild. Skill in nest building and parental care are remarkably developed. The nest building male has the ability to spin a viscid thread from a kidney secretion, which binds the nest of different material together. More than one female may lay eggs in the nest of one male, but only the male remains to guard. The nests are built of various materials and differ in shape and position (Wunder 1930; McInerney 1969; Rowland 1974a). Different species build their nests in a variety of habitats and different species deposit their eggs in a variety of places within it. In order to survive the eggs have to be constantly ventilated by the guarding male. The male guards the young after they hatch (Fig. 13b) until he can no longer orally return the wandering offspring back in the nest. Up to 200 eggs from 5 to 7 females were reported in one nest. One male sometimes guards several nests, skillfully rotating the order of ventilation. This makes the male of the stickleback "about the busiest thing among fishes that can well be imagined." Ventilation is achieved by fanning with the pectoral fins, or by

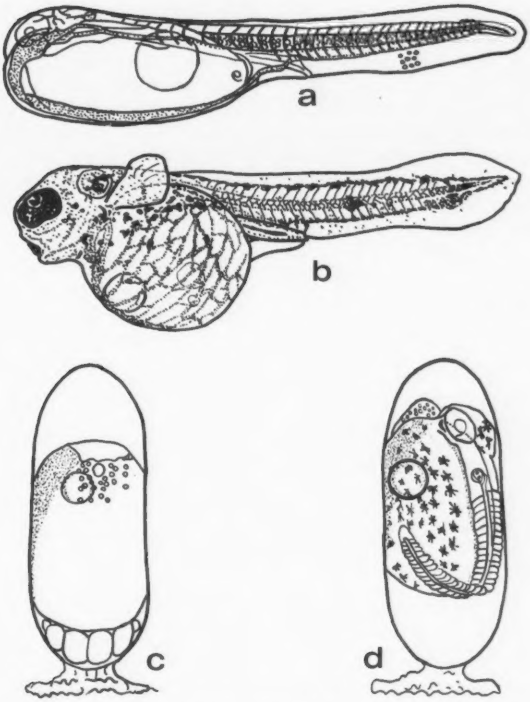

FIG. 13. a, Eleutheroembryo of *Lepomis gibbosus* 3.1 mm long, b, *Gasterosteus aculeatus* 4.3 mm long and eggs of *Amphiprion chrysopterus*, c, after 3 h of incubation in early cleavage phase, d, after 48 h in the middle of embryonic phase. After (a) Balon 1959c, (b) Kuntz and Radcliffe 1918, and (c, d) Allen 1972.

pumping water through the openings of the nest with the mouth, in a sort of speeded-up respiration. The male of *Apeltes quadracus* is reported to ventilate the eggs "by placing his snout into the hole [in the side of the nest] and sucking water through the nest by beating movements of his opercula" (Rowland 1974b).

The only non-stickleback with a similar ability is *Aulorhynchus flavidus*, which utilizes "algal fronds [which it binds] by means of a thread-like secretion in such a fashion that the growing frond tips point downward. ... The eggs are then placed above the place of binding, an activity that would seem to be derived from a former nest-building habit." The eggs, 2 mm in diameter, ranging in color from red to tan, are deposited in more than one cluster and are guarded by the male. Possibly other non-sticklebacks should be included in this guild. The British wrasse, *Crenilabrus melops*, was reported to build elaborate nests of algae (Potts 1973), and can be considered as a potential member of ariadnophils if the use of thread-like secretion is proven.

Canada: *Apeltes quadracus*, *Culaea inconstans*, *Gasterosteus aculeatus*, *G. wheatlandi*, and *Pungitius pungitius*.

Europe: *Gasterosteus aculeatus*, *Pungitius pungitius*, and *P. platygaster*.

Actinariophils (B.2.8) nest under the active protection of the tentacles of sea anemones (e.g. *Radianthus*). An area next to the pedal disc of an actinarian is cleaned, and cylindrical eggs (Fig. 13c, d) are attached to it. Both parents guard intermittently, fan with their pectoral fins and occasionally with their caudal fins, mouth the eggs, and exhibit substrate nibbling at the periphery of the egg clutch, similar to the initial cleaning activity. Dead eggs are eaten. Mouthing may involve coating the eggs with a mucus that protects the eggs against nematocysts. The tentacles of the anemone frequently brush the egg clutch without harming it. Hatching occurs in a week and during the night. The eleutheroembryos first sink to the bottom but then immediately swim up in zigzag fashion to the surface. A phototropic reaction has been observed. Larvae swim near the surface during the daytime and lie on the bottom at night. Soon after this short pelagic mode of life, early juveniles 6–8 mm long select their host anemone and begin their acclimation by careful and brief contacts. At this time they are promptly stung but they dart away. After 24 hours, however, they are able to enter the oral disc of the anemone without harm. Probably in different species (Allen 1972) either the fish or the actinarian becomes acclimated.

Only 26 species of the pomacentrid genus *Amphiprion* are presently known to have this nesting dependency on actinarians.

C. Bearers

EXTERNAL BEARERS (C.1)

External bearers carry their developing eggs on the surface of their bodies or in externally filled body cavities or special organs.

Transfer brooders (C.1.1) encompass fishes that carry their eggs for a certain time before depositing them. In some members of this guild only two to four large, adhesive eggs are released at a time into a "basket" formed by the cupped ventral fins (see photographs in Axelrod 1971, p. 28–34). Female *Callichthys fasciatus* and *Corydoras* sp. carry the spherical eggs in their fins during a prolonged search for a suitable place to attach them. Other members of this guild such as *Cubanichthys cubensis* and *Oryzias* spp. have 20–40 eggs, about 1 mm in diameter, hung in a cluster from the genital pore of the female (see photograph in Axelrod and Shaw 1967, p. 385). Yamamoto (1967) described this condition in *O. latipes* as follows: "After the ova are expelled *en masse* at the time of mating, a cluster of eggs remains attached to the belly of the female for some hours, suspended from the oviduct pore by fine threads attached to the chorion." Some observers noted that the eggs were carried by the female until they hatched; others found that the female sought out convenient notches between a plant leaf and stalk in which to catch and tear off the egg cluster. Nevertheless, in *Corydoras* and *Oryzias* these procedures seem to be an adaptation to removing the eggs from the spawning site, because the attention of predators drawn by the spawning act endangered the eggs. This guild has probably less phyletic significance for the guilds that follow it than has, for example, the hen-like guarding in *Loricaria* (B.1.1) for the skin brooders (C.1.5) and pouch brooders (C.1.6), or the mouth-transfer of eggs by nest spawners (B.2.1) for the mouth (C.1.3) and gill-chamber brooders (C.1.4). However, the possible relationship of the second type of transfer brooders with the forehead brooders cannot be denied.

Forehead brooders (C.1.2) are a unique monophyletic guild. Maturing males of *Kurtus gulliveri* and *K. indicus* gradually develop a hook on the superoccipital region. This hook juts forward and out from the forehead and the tip is bent downward (Fig. 14a). In the spawning season, this appendage is covered with a thick layer of skin. A cluster of eggs, attached by a thread looped through the hook, is carried on the forehead (Fig. 14b). When the eggs are hanging from the oviduct pore, they are probably collected by the male. This situation is similar to that described for the previous guild.

Mouth brooders (C.1.3) incubate their eggs in the buccal cavity and form a diverse polyphyletic guild. Mouth brooding evolved from the substrate guarders' practice of cleaning and taking eggs into the mouth during hatching, and of transferring the brood orally from incubation substrate to nursery pits. Transfer brooders could also have contributed to the development of mouth brooding (Oppenheimer 1970). The female of *Tachyrus argyropleurodon* deposits her eggs in a basket formed by the pelvic fins from which she transfers them directly to the mouth of the male. In *Apogon imberbis* "eggs are cast in a single mass of material bound together by tendrils arising from one pole of the eggs. The male immediately takes them in his mouth. ... The eggs are spat out by the male near the time of hatching." In *Apogon semilineatus*, which practices a similar egg transfer, both sexes brood the eggs orally. The female of *Glossamia gilli* releases her eggs grouped in a thin membrane. The male tears this apart immediately and takes the eggs, one at a time, into his mouth. Evolutionarily, probably only the males developed this habit, and it is still restricted to males among such species as *Bagre marinus*, *Galeichthys felis*, *Netuma commersoni*, *Arius falcarius*, *Potamarius nelsoni*, and *P. izabalensis*.

In true nesting fishes, however, first "both sexes take part in excavating a nest hole for the reception of the eggs, after which the female takes them into her mouth." This is also the case with *Pelmatochromis* spp. In more advanced brooding

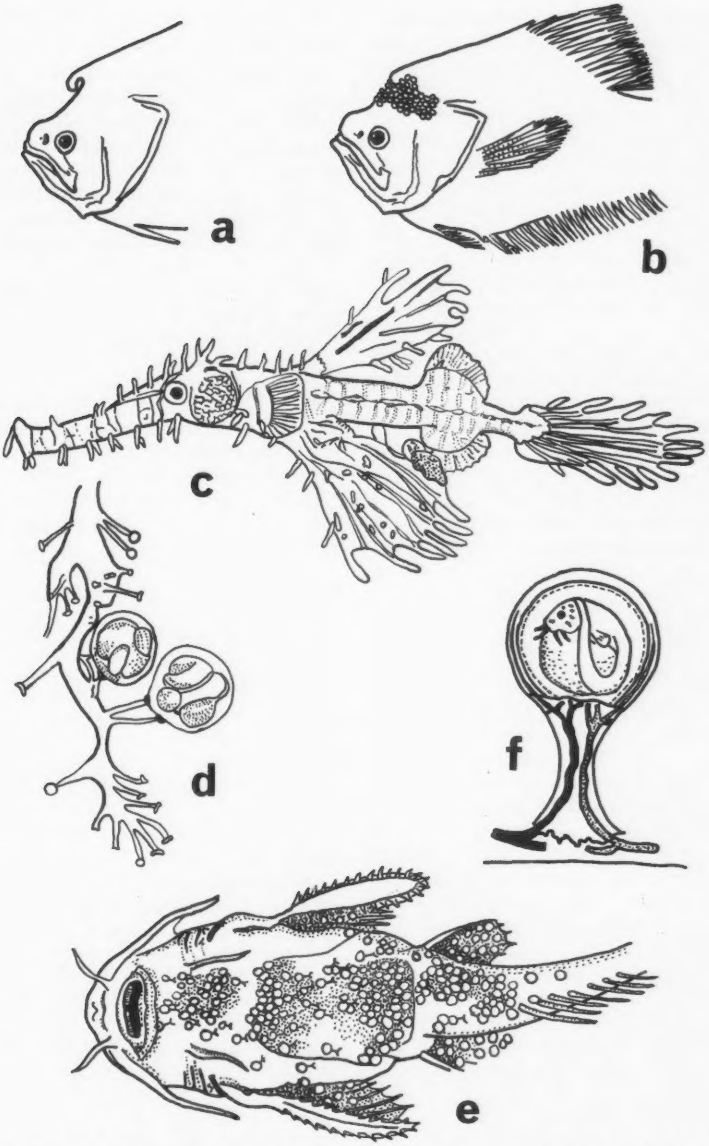

FIG. 14. a, b, Foreheads of *Kurtus gulliveri* males with bare hook (a) and with lodged egg cluster (b); c, d, female of *Solenostomus* (c), detail of pelvic fin of female with attached eggs (d); e, f, ventrum of *Aspredo* female with attached eggs (e) and detail of an egg on stalk within vascularized skin capsule (f). After (a, b) Guitel 1913, (c, d) Willey 1910, and (e, f) Wyman 1859.

strategies in *Haplochromis* spp., *Tylochromis* spp., and some *Sarotherodon* spp., the protective collection of eggs is carried out immediately after deposition, and fertilization is ensured within the buccal cavity. Even then sometimes elaborate, closely guarded sand nests are built (*Haplochromis heterodon*, *Sarotherodon karomo*) by the male. These nests are usually species-specific, circular depressions in the sand ranging from 0.3 to 1 m in diameter. They have conical mounds and various types of rims and eccentric grooves. Smaller nests are excavations produced by fin and body movements. "... the nest is built up by the laborious process of moving mouthfuls of sand into the correct position, and by this method some of the most elaborate and beautiful nests are constructed" (Fryer and Iles 1972).

Males of these species possess bifid genital tassels or pigment egg-dummies on the anal fin, which stimulate females to inhale sperm before and after egg laying and egg pickup. The same is true for the free spawning (without a nest) *Tropheus* whose eggs can be moved immediately to the protection of the mouth without losing time in depositing them in a nest for external fertilization. This is vital in a crowded habitat, or where egg-eating specialists are never far away (Fryer and Iles 1972).

Mouth-brooding individuals can usually be recognized by a marked downward deflection of their buccal cavity. The eggs are few but large. *Tropheus moori* and *Labeotropheus trewavasae* in my aquaria carried only two to eight eggs per female and it is unlikely that in nature they can brood more than 20 eggs. The eggs are spherical or elliptical, with a maximum length of only 3 mm in some species but as large as 7 mm in *T. moori* and *Hemibates stenosoma*. *Osteoglossum bicirrhosum* carries about 20 eggs 6 mm in diameter, whereas males of *Bagre marinus* carry over 50 eggs of about 20 mm in diameter, a size also reported for *Arius dispar*.

The yolk in the eggs of most mouth brooders is very concentrated; water content is only 50–60%. Early in the embryonic phase a strong blood circulation develops with networks of respiratory vessels spread out in the dorsal and anal fin folds and on the yolk. After hatching, the egg membranes are usually swallowed by the brooding parent. Except in *A. imberbis*, the larvae are brooded to the end of larval period (if such exist), and only the relatively large, free swimming pterygiolarvae or juveniles (like those in salmonids) are released from the mouth. With some exceptions, the young periodically seek refuge in their mothers' or fathers' mouths, and they are herded for some time. No cement glands are present.

None are known from freshwaters of Canada or Europe.

Gill-chamber brooders (C.1.4) may be derived from mouth brooders; they include, so far as is known, only the North American cavefishes. *Typhlichthys subterraneus*, *Amblyopsis spelaea*, *A. rosae*, *Speoplatyrhinus poulsoni*, *Chologaster agassizi*, and *C. cornuta* incubate their eggs in their gill chambers. The female probably takes the eggs into her mouth and subsequently passes them into the gill cavity, where they go on developing until some time after hatching. In the end, the gill cover muscles usually slacken and the young fall out. About 70 eggs 2.3 mm in diameter are reported per female.

Skin brooders (C.1.5) are a specialized guild. Females in some species or males in others carry the eggs attached to the ventral surface of their body. Females of a group of South American catfishes, which includes *Bunocephalus* spp., *Aspredo* spp., *Platystachus* spp., and *Loricaria piracicalae* develop a special, differentiated, spongy skin on their ventrum. These females roll on the eggs after they have been fertilized and the eggs adhere to the skin. After some time a cup with a stalk envelops each egg. This cup, which grows from the skin, is vascularized (Fig. 14e, f) and performs some kind of gas and nutrient exchange. This type of reproductive strategy is also used by some pipefishes, in which females loosely attach groups of eggs or single eggs to the ventral surface of the male during copulation. The eggs become embedded in a spongy mass on the males' ventrum (*Entelurus aegnoreus*, *Nerophis*, *Phyllopteryx*) or attached singly to special stalks. Skin incubation probably gave rise to the next guild (Fig. 14c, d).

Pouch brooders (C.1.6) encompass the same phyletic lines as skin brooders. The eggs, similarly incubated on modified skin, are enveloped by specially transformed structures, or covered with a membrane or plates, thus forming a marsupium. In *Loricaria vetula* and *L. anus* the pouch is formed by an enlarged and everted lower lip of males. In females of *Solenostomus* spp. the very large ventral fins "with their inner margin adnate to abdomen, their outer margins united at their base for a short distance, [form] a large pouch for the reception of the eggs, which are fastened by peduncles on threadlike filaments of the abdominal skin" (Weber and de Beaufort 1922). In pipefishes and seahorses (*Syngnathus*, *Siphistoma*, *Penetopteryx*, *Stigmatophora*, *Hippocampus*) a special marsupium is formed on the ventrum of the males. The brood pouch can be membranous or covered by protective bony plates. In some, the eggs are embedded in a spongy skin covered only by lateral protective plates (see photograph in Axelrod and Shaw 1967, p. 86). The eggs are deposited in the pouch during mating when both partners embrace face to face. The protruding oviduct of the female is "thrust into the buttonhole-shaped opening at the anterior end of the marsupium"; the eggs are fertilized as they pass into the pouch. About 150 eggs can be incubated. One female fills up the pouches of more than one male, or one male collects eggs from several females if his pouch has not been filled by the first female. The male is capable of pushing eggs to his pouch by peristaltic motions. The same mechanism is also used to flush out the empty pouch before new eggs are accepted. The offspring leave the pouch late, as juveniles.

None occurs in freshwaters of Canada; in Europe: *Syngnathus abaster* and *S. nigrolineatus*.

INTERNAL BEARERS (C.2)

The eggs are fertilized internally before they are expelled from the body cavity. Special organs are sometimes (or ? usually) developed to facilitate sperm transfer. Mating does not necessarily coincide with fertilization. After an initial copulation, sperm can be stored for a lifetime. After fertilization the eggs can be expelled and incubated externally or retained in the body cavity of the female, after which full-grown juveniles are born. Because an excellent review has recently been published by Hoar (1969), only the most basic data will be given in the following text.

Ovi-ovoviviparous (C.2.1) fishes, which form this intermediary polyphyletic guild, lay eggs fertilized internally. Egg expulsion can take place at the moment of fertilization, at the beginning of cleavage, or at any time during the embryonic phase. Internal fertilization is effected by special, often complicated, organs. In *Corynopoma riisei*, *Glandulocauda inequalis*, *Coelurichthys microlepis*, *C. tenuis*, and *Gephyrocharax valencia*, it has been suggested that "sperm vesicles are lodged in the female's oviduct" without a copulatory organ and "spawning occurred in the absence of males" (Nelson 1964). In elasmobranchs the eggs are internally fertilized with the help of large mixopterygia. After fertilization the eggs are coated in a durable horny case. In *Heterodontis francisi* and *H. galeatus*, for example, the eggs have the shape of a capsule with a double spiral; *Rhincodon typus*, *Scyliorhinus canicula*, *Galeus melanostomus*, and *Raja* spp. produce squarish eggs with long spiral tendrils at each corner. In some species these tendrils entangle objects and assist in further expulsion of the egg. In *Ginglymostoma cirratum* the horny egg capsule ruptures long before the expulsion of the embryos. The embryos have external gill filaments and can develop normally in sea water if surgically taken out of the female. Incubation is usually long, 10 or so weeks, and the number of eggs very low. Twenty-six young were reported from a female of *G. cirratum*. *Chimera* spp., *Callorhynchus*, and *Hydrolagus* have an additional frontal clasper and eggs in a horny case with two lateral fins.

Males of *Trachycorystes striatulus*, *Asterophysis* spp., and *Tatia* spp., for example, have a fleshy intromittent organ. Eggs are fertilized after ovulation and before they are extruded. *Phenacostethus smithi* has a complicated fleshy copulatory organ called a priapium, which in *Gulaphallus mirabilis* is assisted by special grasping organs, the toxactinium and the ctenactinium. Recently, an entirely different copulatory organ has been described in *Pantodon buchholzi*, a fish that has for some time been known to deposit internally fertilized eggs (Lastein and van Deurs 1973). *Tomeurus gracilis* has an elaborate gonopodium. The female deposits eggs, always attaching them to some object by long filaments on the chorion. The eggs can also be incubated internally. In either external or internal incubation, the embryos of this species develop a normal pericardial serosa that completely envelops the head and that is similar to the true livebearing *Xiphophorus* or *Gambusia*. *Horaichthys setnai* produces similar embryos from eggs 0.21 to 0.35 mm in diameter after 10–20 days of incubation. The genital pore of the female, however, is deflected to one side matching a similar deflection of the gonopodium in the male.

Ovoviviparous (C.2.2) fishes incubate their eggs in the body cavity where they grow by utilizing the yolk. Fertilization is internal and is usually facilitated by intromittent organs. "*Latimeria* lacks pelvic claspers or anal fin specialized as an intromittent organ ..., but males do possess modifications of the cloacal region, which are possibly developments for internal fertilization. These are the extension of the cloaca into a tubercle, and a series of surrounding erectile caruncules" (Griffith and Thomson 1973).

The deposition of eggs or embryos in members of this guild is delayed until hatching or even beyond that. Radioisotope studies have proved that nutriments are not taken by embryos from the female (*Scyllium*) during incubation and gestation in her body. Well-formed egg cases with rudimentary tendrils are often incubated in the female body for a year or up to 22 months

(*Chlamydoselachus anguineus*, *Hexanchus griseus*, *Notorynchus maculatus*, *Scapanorhynchus owstoni*, *Squalus* spp., etc.). It is reported that a female elasmobranch can have from 2 to over 100 embryos. The eggs are large and single or up to four or five per case, and the case is sometimes divided into chambers (*Pristis* sp.).

On January 5, 1972, a female of *Latimeria chalumnae*, 1.63 m and 78 kg, was landed at Anjouan with 19 eggs 85–90 mm in diameter (Anthony and Millot 1972). "We agree that this evidence makes the occurrence of viviparity in *Latimeria* unlikely, but we feel that there is a strong possibility that the living coelacanth is ovoviviparous, providing an ample supply of nourishment prior to fertilization but maintaining the embryo internally during early development. ... Finally, we note that the very size of *Latimeria* eggs is a strong argument that this species has a high degree of parental care, involving either ovoviviparity or guarding a cluster of eggs. It is difficult to believe that *Latimeria* could survive at all unless the small number of eggs were protected from predation" (Griffith and Thomson 1973).

Sebastes marinus expels the embryos just before or after they hatch from eggs 0.18–0.38 mm in diameter. The eleutheroembryos are poorly developed. The deep water brotulid *Cataetyx memorabilis* hatches at the time of parturition (Meyer-Rochow 1970), while another brotulid *Dinematichthys ilucoeteoides* was reported to hatch within the ovary long before parturition (Wourms and Bayne 1973). *Xenopoecilus poptae* from Celebes (= Sulawesi) lakes is an exemplary type for this guild. "As soon as the eggs are extruded, the young fry is hatched and swims with the mother. In the period of [parturition], broken egg membranes, rising to the surface, cover extensive stretches" (Weber and de Beaufort 1922). Another pelagic genus *Comephorus* of Lake Baikal has very similar parturition. In contrast to the other members of this guild, and the members of the following guild, *Comephorus* is known to produce large quantities of eggs per female.

Viviparous (C.2.3) fishes produce embryos whose partial or entire nutrition is supplied by the female via special absorptive organs (Turner 1940a, b, c, d), i.e. endogenous nutrition from the yolk is supplemented already in the prenatal phase by exogenous nutriments from the mother (Amoroso 1960; Hoar 1969). Fertilization is internal, but one group of these fishes lacks intromittent organs. *Carcharias taurus* seems to produce one young at a time, which is free within the body cavity and feeds on eggs gradually produced by the female. A large experienced predator is born. In general, however, an intimate association develops between the uterine wall of the female and embryos and 10–70 placental young per litter are born. An extended gestation period of up to 12 months was reported in elasmobranchs (Matthews 1950) that have these adaptations: *Carcharhinus milberti*, *Prionace glauca*, *Galeocerdo cuvieri*, *Mustelus canis*, *Eulamia floridana*, to mention only a few.

No more than 50 young in a litter are born to livebearing teleosteans and gestation does not, in general, take longer than a month. Males of many species including *Goodea luitpoldi*, *Neotoca bilineata*, and *Hubbsina turneri*, do not possess intromittent organs, whereas all the other members of this guild have claspers, clasper-like penises, or complicated gonopodia. Nutriment absorption and gas exchange from the female is achieved through the transformed embryonic fin fold or special processes (trophotaeniae), which the embryos develop for this purpose (Fig. 15a, b, c). The poeciliids are best known for having unchorionated eggs and superfetation, i.e. eggs without egg membranes, and the ability to carry several litters at different stages of development instead of a single litter at a time. Here, also, a special pericardial serosa (*Poecilia*, *Xiphophorus*, *Gambusia*, etc.) replaces the absorptive processes (*Parabrotula plagiophthalmus*) for the absorption of nutriments from the mother (Fig. 15d). This serosa can, in some, completely envelop the head. Fishes of different phyletic origin and with different adaptations for embryonic exogenous nutrition belong to this guild.

Discussion and Conclusions

The exploitation of ecosystem resources is dependent entirely on the density of each exploiting fish taxon. In a natural multispecies system, the available food resources, if left unutilized by the disappearance of one taxon, are eventually utilized by another. This is, in the long run, reflected in much the same total production within the same nutrient load, irrespective of the fate of single fish taxa (Balon 1974). Furthermore, a single taxon of fish can change readily from one feeding habit to another, which is known as changes from one type of "population dynamics" to another as reported earlier (Balon 1963). Even changes in skeletal structures are reported to accompany changes in food (Greenwood 1965) and extreme specialists like eye-biters, fin-eaters, or scale-eaters (Roberts 1970; Fryer and Iles 1972) are now known to be exceedingly inconsistent in their food preference. In my aquaria they in general

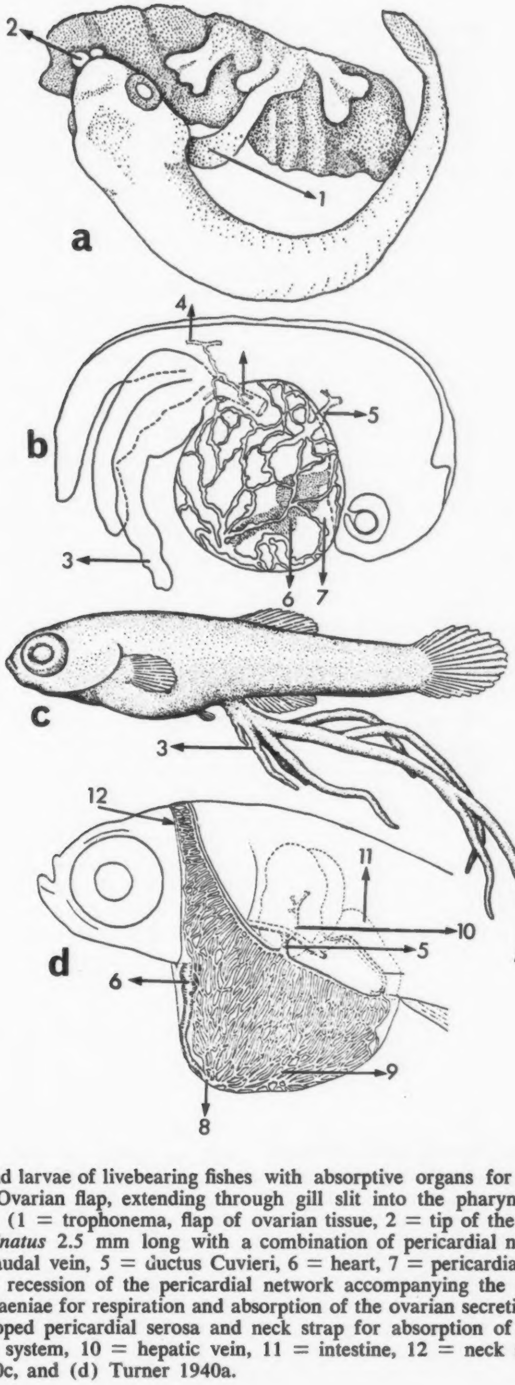

FIG. 15. a–d, Embryos and larvae of livebearing fishes with absorptive organs for "placental" nutrition: a, larva of *Jenynsia lineata.* Ovarian flap, extending through gill slit into the pharyngeal and buccal cavities, protrudes from the mouth (1 = trophonema, flap of ovarian tissue, 2 = tip of the ovarian flap), b, embryo of *Girardinichthys innominatus* 2.5 mm long with a combination of pericardial network and trophotaeniae (3 = trophotaenia, 4 = caudal vein, 5 = ductus Cuvieri, 6 = heart, 7 = pericardial sac), c, larva of *Zoogonecticus cuitzooensis,* after recession of the pericardial network accompanying the yolk sac absorption, uses highly vascularized trophotaeniae for respiration and absorption of the ovarian secretion, d, embryo of *Poecilia reticulata* with fully developed pericardial serosa and neck strap for absorption of ovarian secretion (8 = sinus venosus, 9 = portal system, 10 = hepatic vein, 11 = intestine, 12 = neck strap). From (a) Turner 1940d, (b, c) Turner 1940c, and (d) Turner 1940a.

TABLE 2. Number of indigenous freshwater fish species per guild in different faunae. References at right are the sources of the primary faunal lists and numbers of species.

	A.								B.									C.	
Guilds	1. 1	1. 2	1. 3	1. 4	1. 5	1. 6	2. 1	2. 3	1. 1	1. 2	1. 4	2. 1	2. 2	2. 3	2. 5	2. 6	2. 7	1. 6	
Arctic Basin	3	3	15	6	3	2	4	—	—	—	—	1	—	—	4	—	2	—	Scott and Crossman 1973
Pacific Basin	1	3	22	3	2	2	10	—	—	—	—	—	—	—	6	—	1	—	
Hudson Bay Basin	4	5	20	8	7	5	10	—	—	—	—	8	2	—	10	1	3	—	
Atlantic Basin	8	11	25	18	17	6	11	—	—	1	—	10	3	—	13	1	5	—	
Canada	8	13	43	20	18	8	21	—	—	1	—	10	3	—	17	1	5	—	
British Isles	5	5	4	6	5	5	4	—	—	—	—	—	—	—	2	—	2	—	Maitland 1972
Vistula River	4	4	10	7	10	3	3	1	—	2	—	—	1	—	2	—	2	—	Balon 1964b
Danube River	5	7	11	11	13	5	5	1	4	3	—	1	2	—	2	—	2	1	Balon 1967b
Europe	13	14	41	23	29	12	10	1	8	6	—	2	4	—	11	—	3	2	Banarescu et al. 1971
Amur River	20	2	9	—	9	8	6	3	—	3	1	—	—	1	7	—	2	—	Kryzhanovsky et al. 1951

take any available food; thus, any given feeding habits seem to be retained only within a given taxocene (Chodorowski 1959), density, and environmental conditions. Therefore, from the point of view of resource exploitation, factors other than the feeding habits are more important in governing density, biomass, and production. In this sense reproductive guilds of fishes are more meaningful than feeding strategies or trophic guilds (Root 1967, 1973), the latter being actually a supplement to and a part or reflection of the first. Accordingly, sex ratio as well as occasional hermaphroditism, protandry, and protogyny will be additional adaptations related in a more important way than feeding to reproductive strategies and potential population density (Warner 1975a, b).

Most likely the importance of reproductive guilds in other animals will be similar in importance to the reproductive guilds in fishes. A convincing example is the increase of the Canada goose density by the construction of man-made breeding platforms in the prairie provinces (A. Macpherson personal communication). Largest mortality has been reported to occur during the early life of fishes, in the embryonic and larval periods (Marr 1956; Vladimirov and Semenov 1959; Balon 1960). This leaves us, at least in fishes, with the periods of life where the density is decided, and only these periods should be essential for ecological classification.

The ideal situation would now be to use the proposed concept in quantitative ratios between single guilds, in aggregated values of density, biomass or, ultimately, ecological production, similarly to earlier attempts (Balon 1966). A valid pattern should emerge, and if related to known stresses, could uncover important features of ecosystem dynamics (Smith 1968; Christie 1974), and may facilitate better (Ryder et al. 1974) understanding and construction of predictive models (Regier and Henderson 1973; Regier 1974). While data needed for such an approach are very scarce, let me in this introductory paper apply the guild concept again only qualitatively (Kryzhanovsky 1949; Balon 1962b).

The 32 guilds presented here encompass all reproductive systems known in fishes at the present time. Some unknown systems may still be discovered and the number of guilds increased, or a better understanding of some ecomorphological and physiological features of early development may cause a split in present guilds and have the same effect ultimately. In single ecosystems we shall always deal with a much lower number of guilds.

Marine systems are very different from freshwater ones. In marine systems more fishes represent guilds of guarders, bearers, and nonguarding pelagophils, whereas the majority of freshwater fishes are concentrated in guilds of nonguarders excluding pelagophils (Kryzhanovsky 1949). Similar differences seem to apply to fluvial versus lacustrine systems and to temperate versus tropical systems.

In a number of guilds (Table 2) the freshwater faunae of Europe and Canada are very much alike. The lowest number — nine guilds — occur in the British Isles and the Pacific Basin of Canada. Of the 15 guilds representing the fishes of the whole of Europe, the Vistula River has 11 and the Danube River has 14 guilds. The Arctic Basin has 10, the Hudson Bay Basin 13, and the Atlantic Basin 14 of the 14 guilds that encompass the whole freshwater fauna of Canada.

Canadian and European pelagophils are fish mainly of marine origin, whereas those in the Amur River (selected for contrast and available data) are of freshwater origin; the ratio is 8 Canadian to 12 European to 19 Amurian. Most likely geochronological sequences are reflected in these differences. Invasion routes and the different histories of survival in the geological succession of ecosystems probably played a no less important role. The evolutionary significance, for example, of the very high number of open substratum spawning and hiding lithophils in Canadian (Table 2) as compared to European waters has as yet to be understood. The same applies to guarding speleophils and the reversed numbers to guarding lithophils. All these highly significant differences certainly have a historical meaning (McPhail and Lindsey 1970) and will hopefully be correctly interpreted when more ecosystems are analyzed and compared from an historical point of view.

I have no doubt that the species in guilds of guarders and especially of bearers evolved from species with much simpler nonguarding systems. Obvious examples of such evolution in more advanced guilds were given previously. For each of them special ecosystem conditions must have been available, but the length of geological time available seems to be important. The species in the more complex guilds of guarders and bearers had to have a longer time to achieve their level of adaptation and, in general, are limited to more specific ecosystems and taxocenes (Hutchinson 1967). This availability of evolutionary time applies to the consecutive guilds along each section in the ecological classification, as well as to the entire classification. Specialization to a limited ecosystem and taxocene is also reflected in decreased ability to invade new areas. The "primitive" species in section A are the first to follow the retreat of the ice sheet; livebearers never managed to move far from very old biotopes. But in this respect ariadnophils form an exception to the rule.

The terms "primitive" and "complex," however, are rather conjectural. The morphophysiological adaptations in reproductive systems of species in A are as complex as those of species in B and C. The functions of a morphological isolatory mechanism (gonopodium) in C has a counterpart in A in the elaborate prespawning and spawning rituals (Balon 1956; Fabricius 1959; Fabricius and Gustafson 1958). The ecomorphological adaptations are interwoven with ethological adaptations throughout all reproductive systems. Where the one is missing the other is more intense.

For most species, placement in a certain guild is highly conjectural because of lack of relevant data. For some faunae placement is presently impossible. However, the needed data are accumulating rapidly and I intend, in the years to come, to elaborate further upon this ecological classification of fishes.

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BOOK REVIEWS/COMPTES RENDUS

Netting Materials for Fishing Gear. *Gerhard Klust. Fishing News (Books) Ltd., by arrangement with FAO, Surrey, England, 1973, 173 p. £2.60.*

The subject of this FAO Fishing Manual is basically textile technology as applied to twines (yarns) and netting used in commercial fishing gear. Much of the information has appeared in print before, but mostly in relatively obscure papers and reports not generally available to the fishing and textile industries. Thus, it is gratifying to see this material collected by an expert into one handy volume.

The presentation is very good, particularly considering that it is not in the author's native language. However, in all fairness to the author and in view of the tremendous amount of work required to prepare the manuscript, Anglophone editing should have been done more carefully. For example, on page 134 "rentability" refers to ease of rending (tearing), not to renting or leasing. Also, there are many passages which, though correct, could be rephrased into a more comprehensible form; and there are many mechanical errors such as reference on page 12 to "underlined" items in Table 1 which turn out to be italicized.

The discussion of twine constructions and properties, though extensive, would have been clearer if six, rather than four, basic fiber types had been recognized. "Continuous filaments" should be more accurately referred to as continuous mutlifilaments or simply multifilaments. After all, monofilaments are continuous too. In discussions of monofilament netting yarns (twines) the reader is repeatedly forced to cross-check whether these are single or folded. There are two types of monofilament netting yarn and the qualifying adjective should be used generously. An important fiber type for hanging twines in particular, which was ignored completely, is the textured continuous multifilament. It has the knot stability of staple-fiber yarns, but with greater strength and higher modulus.

Two mechanical considerations should be corrected. In at least two places reference is made to treating multifilament netting with tar or resin to increase the density. It is not possible for tar with a density of about 1.1 g/cm^3 to increase the density of PA (1.14 g/cm^3) or PES (1.38 g/cm^3). After thorough soaking, tarred netting is slightly lighter in water than is the same dry weight of untreated PA or PES netting. For purse seines, the netting must fall through the water before the air between the fibers has time to be displaced by water. This entrained air provides a buoyancy that decreases sinking speed. The function of the tar is to exclude interfiber air thereby avoiding the unwanted, temporary buoyancy.

In the field of textile mechanics the cross-sectional area of the fibers in a yarn is very difficult to determine, and it does not relate in a consistent way to the thickness (diameter) of the twine or cordage. Many fibers have very irregular shapes (even the round ones are seldom truly circular) and most fibers change cross-sectional area considerably with changes in humidity and stress. Furthermore, the strength of staple yarns depends as much on interfiber friction as on fiber strength. Therefore, by contrast with solid structural materials, the tensile strength of textile yarns is virtually never expressed as force per unit cross-sectional area of fiber. It is recommended that, in keeping with common practice, tensile strength (page 60) be given the same definition as breaking load i.e. total load to cause rupture.

Tensile stress, on the other hand, is force per unit area by traditional definition. The effective breaking stress (kgf/mm^2) on the fiber may be calculated as the product of tenacity (gf/tex) and fiber density (g/cm^3), rather than by the dimensionally incorrect relation in the manual (page 60), but this is a theoretical figure and is much inferior to tenacity itself for comparing the strengths of different textile materials. Breaking length (numerically equal to tenacity) is a useful concept that is better than breaking stress (kfg/mm^2) for comparing the strengths of netting yarns made of different kinds of fiber, particularly when goods are considered by weight or mass.

The manual (page 147) refers to the size (length or opening) of "stretched" meshes, contrary to preferred use of the adjective "extended" in International Standard ISO 1107–1974 and bibliographic reference 54. "Stretch" is "make taut, tighten," implying a tensile load. In section 3.2.2.3. "fully stretched netting" means the netting is loaded to the breaking point. By contrast "extend" is "lay out at full length" — surely the meaning intended in the manual.

The discussion of elasticity should consider time-dependent elasticity in apposition with immediately recoverable elasticity and permanent elongation under load, rather than identifying it as an afterthought. Time-dependent elasticity is a very important textile property in fishing gear behavior. Incidentally, in Fig. 53, the immediate recovery is represented by the straight lines from d to e, not from b to c as stated in the text.

The manual is purported to deal only with "netting yarns and not with . . . the properties of netting and fishing nets." By this policy, the final, essential link between the textile industry and the fishing industry is omitted. Some alleviation is attempted in Chapter 3 on "Choice of netting materials . . . ," but the discussion devolves primarily by induction from observations in the textile laboratory rather from experience in the fishery. For example, the manual recommends large elongation for netting in trawls in order to maximize toughness. However, except in the cod-end, meshes are made as large as practical to minimize drag, visibility, noise, etc. If the mesh size is subject to large, elastic increase under load, fish will become caught in the netting, particularly in the bellies and intermediate, creating a great deal of unnecessary work and lost fishing time.

These criticisms do not detract from the value of the manual. It is a very useful and comprehensive reference as it stands. However, when time comes for a second edition, it should be revised, preferably by experts in the various basic disciplines involved.

Considering the approach and scope, this manual will be most useful to textile technologists involved with fishing gear materials. It will be useful also to more advanced fishing gear technologists in the design and construction of fishing nets. It contains many basic concepts and much information essential to a proper understanding of fishing gear behavior as a factor in fishing efficiency and fishery management. Dr Klust is to be thanked for a very worthwhile contribution.

P. J. G. Carrothers

Department of the Environment
Fisheries and Marine Service
Biological Station
St. Andrews, N.B. E0G 2X0

(R10)

Modern Inshore Fishing Gear — Rigging and Mending. *John Garner. Fishing News (Books) Ltd., Surrey, England, 1973, 67 p. £3.75.*

This is the fourth, practical fishing gear book by Mr Garner, and it maintains the high and useful standard of his previous publications. It is addressed primarily to the British inshore trawler fleet, but with a little translation of indigenous terms is readily understood by a much wider audience. This manual for learning net hands covers the subject comprehensively and hence should be useful also to marine biologists in search of sampling gear.

The declared purpose is to show advances made in the design, rigging, and setting of nets by the use of man-made fibers. Undoubtedly the most recent practices in design and rigging are described, but their evolution from the introduction of man-made fibers has not been made clear. Light-weight nylon twines have been specified for some trawls, but there is little discussion of the unique properties of man-made fibers for fishing gear. Also, virtually no space has been given to describing setting and hauling procedures.

The discussions of reasons for different trawl shapes, modifications for rough ground and high headline, and variations in door rigging are brief and to the point.

The specifications for the inshore nets require some interpretation but they are usually quite definitive and provide enough information for persons familiar with the principles of trawl design and construction. I believe that in Fig. 11 the upper wing should be 33, rather than 43, meshes deep. The appendix on the method for specifying trawls relates exclusively to the use of handmade netting. The increasing use of machine-made netting warrants similar instructions for specifying trawls made of this material, in a second appendix to avoid confusion.

The topics of fitting and rigging, net braiding, and net mending and assembly have been covered elsewhere in the literature, but they belong also in this manual on inshore trawls. Except for a couple of poorly placed illustrations, this exposition is remarkably easy to understand. The illustrations are good and are used effectively by a brief text in welcome contrast to the more usual practice of indulging in long, complex, virtually incomprehensible, even though correct, dissertations. If you have trouble following other books on these topics, take a look at this one.

Mr Garner discusses a fairly restricted field but he discusses it well. This should be a useful volume for anyone interested in trawl nets.

P. J. G. Carrothers

Department of the Environment
Fisheries and Marine Service
Biological Station
St. Andrews, N.B. E0G 2X0

(R11)

The Poisons Around Us. Toxic metals in food, air, and water. *Henry A. Schroeder. Indiana University Press, Bloomington, Indiana. 1974. 144 p., $8.00.*

This is a useful book about metals. The subtitle really should have been the title. The book is packed with information — a handbook with an interesting story line. It is useful for professionals working with metals, and also for any educated person who wants guidance through the contradictory stories about poisonous metals. There are many useful tables: industrial consumption of metals; amounts entering the environment and where they go; dietary needs; etc.

Schroeder knows his stuff. Recently retired, his Trace Element Laboratory in New England put out more than 100 papers in 20 years, and they are leading works. Now Schroeder has put together his knowledge, and delivers several important messages in plain language, sometimes even blunt language. For example, he describes air quality standards. The U.S. E.P.A. "farmed out the job. ... It was quite clear that the Mitre Corporation, which got the contract, knew less than nothing about the subject ... standards were proposed for eighteen metals ... these limits are unsafe, and show the abysmal ignorance of the men who proposed them." (More fortunately, U.S. drinking water standards "are now quite acceptable except for cadmium"). Another blunt example comes after a detailed discussion of dietary needs: "the Food and Nutrition Board, which was designed to protect our health, is not wholly incompetent to do so ... only 20 out of 24 things. ... A batting average of .167 ..."

The first few chapters deal with general aspects of metals. Much of it is interesting and useful to any scientist. Then there are three chapters on lead, cadmium, and mercury. The disturbing social costs of lead pollution are detailed, along with the dismal behavior of the lead and gasoline industries. Of considerable interest to fisheries workers, Schroeder fearlessly tackles the "great mercury paranoia." He marshals data and draws conclusions which should be good for some argument. He believes that mercury in swordfish is not methylated, and suggests that the Swedish analytical method may create methyl mercury. "... the conclusion is inescapable that ... mercury ... in ocean fish is natural ... has been there since the year one ... we can eat our game fish without harm or alarm."

There is a lot of information on disease and diet which could be profitably read by anyone. Much of it was new to me. Schroeder claims that trace elements are directly involved with 71% of disease deaths in the U.S. He makes a convincing case for the role of cadmium in deaths from hypertension, heart and artery disease, and despairs that his medical colleagues will probably take 15 years to use the remedy he suggests. Of interest in Canada, regions of soft corrosive water have two or three times as many heart attacks as hardwater regions, and Schroeder suggests water treatment as a remedy. He shows a table of the micronutrients lost in refined foods, concludes that chromium is needed to prevent atherosclerosis, and points out that chromium is virtually prohibited as a dietary supplement by U.S. F.D.A. He advises us to supplement our empty diets with vegetables and "seeds ... unrefined except for grinding."

In a final important chapter he tells *what to do* about metal pollution.

There is also a lot of interesting trivia, e.g. the possible cause of Napoleon's death in captivity.

There are few noticeable errors. The organization is somewhat random in the first parts, because of the semipopular style. This is a bit of a problem for the scientist since the index is badly underdone in places. However, such minor problems are far outweighed by the overall worth of the book. You will be doing a service if you get your institutional and public libraries to order copies.

John B. Sprague

Department of Zoology
University of Guelph
Guelph, Ont. N1G 2W1

(R25)

Books Received / Livres reçus

February 1975–March 1975

Algal Physiology and Biochemistry. *Botanical Monographs, Volume 10. Edited by W. D. P. Stewart. University of California Press, Berkeley, 1975. xi + 1003 p. $40.*

Coupling of Land and Water Systems. Ecological Studies 10. *Edited by Arthur D. Hasler. Springer-Verlag, New York, Heidelberg, Berlin, 1975. xvi + 309 p. $24.80.* (Introductory examination of bioenergetics and nutrient cycling as a framework for better understanding of the physical and biological interactions of land and water ecosystems.)

COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Foreword **W. R. Martin**
Preface **E. D. Mitchell**

Meeting Report

Report of the Meeting on Smaller Cetaceans, Montreal, April 1–11, 1974, Subcommittee on Small Cetaceans, Scientific Committee, International Whaling Commission

Invited Papers

Supplementary information on minke whales (*Balaenoptera acutorostrata*) from Newfoundland fishery **E. D. Mitchell and V. M. Kozicki**
Biological parameters of the Antarctic minke whale (*Balaenoptera acutorostrata*) at the virginal population level **S. Ohsumi and Y. Masaki**
New records of the pygmy right whale, *Caperea marginata*, from South Africa, with comments on distribution, migration, appearance, and behavior **G. J. B. Ross, P. B. Best, and B. G. Donnelly**
Autumn stranding of a northern bottlenose whale (*Hyperoodon ampullatus*) in the Bay of Fundy, Nova Scotia **E. D. Mitchell and V. M. Kozicki**
The narwhal, *Monodon monoceros*, in eastern Canadian waters **A. W. Mansfield, T. G. Smith, and B. Beck**
Identity, abundance, and present status of populations of white whales, *Delphinapterus leucas*, in North America **D. E. Sergeant and P. F. Brodie**
Status and aspects of the life history of the bottlenose dolphin, *Tursiops truncatus*, in Florida **D. K. Odell**
Distribution and differentiation of populations of dolphins of the genus *Stenella* in the eastern tropical Pacific **W. F. Perrin**
Ecological aspects of smaller cetaceans, with emphasis on the striped dolphin (*Stenella coeruleoalba*) **M. Nishiwaki**
Progress report on the biology of the Franciscana dolphin, *Pontoporia blainvillei*, in Uruguayan waters **R. L. Brownell**
Preliminary notes on the occurrence and exploitation of smaller Cetacea in Greenland **F. O. Kapel**
Preliminary report on the Norwegian fishery for small whales: expansion of Norwegian whaling to arctic and northwest Atlantic waters, and Norwegian investigations of the biology of small whales **I. Christensen**
Relation between stranding mortality and population abundance of smaller Cetacea in the northeast Atlantic Ocean **S. G. Brown**
Long-finned pilot whales, *Globicephala melaena*, driven ashore in Ireland, 1800–1973 **C. E. O'Riordan**
Dolphin and small whale fisheries of the Caribbean and West Indies: occurrence, history, and catch statistics — with special reference to the lesser Antillean island of St. Vincent **D. K. Caldwell and M. C. Caldwell**
Review of Japanese small-type whaling **S. Ohsumi**
Progress report on small cetacean research in Chile **A. Aguayo L.**
Modified Leslie–DeLury population models of the long-finned pilot whale (*Globicephala melaena*) and annual production of the short-finned squid (*Illex illecebrosus*) based upon their interaction at Newfoundland **M. C. Mercer**
Preliminary report on the former net fisheries for *Tursiops truncatus* in the western North Atlantic **J. G. Mead**
Investigation of small whale hunting in northern Norway, 1964 **D. C. Foote**
Techniques of live capture of smaller Cetacea **E. D. Asper**
Review of the live-capture fishery for smaller cetaceans taken in southern California waters for public display, 1966–73 **W. A. Walker**
Live-capture killer whale (*Orcinus orca*) fishery, British Columbia and Washington, 1962–73 **M. A. Bigg and A. A. Wolman**
By-catches of harbour porpoise (*Phocoena phocoena*) in salmon driftnets at West Greenland in 1972 **W. H. Lear and O. Christensen**
Incidental catch of cetaceans with salmon gillnets **S. Ohsumi**
Marking of small cetaceans using "Discovery" type whale marks **S. G. Brown**

RECENT PUBLICATIONS / RÉCENTES PUBLICATIONS

The number following each title in each series refers to the establishments listed at the end of the section.

Les nombres qui suivent les titres de chacune des séries désignent les établissements suivants à la fin de la section.

Recent Studies

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles on investigations published in other periodicals, which henceforth may be obtained in reprint form only from the author or originating establishment.

Récentes études

Jusqu'en 1970, ces articles étaient réimprimés, puis reliés dans un volume annuel d'études, comprenant deux parties. Les études de 1969, partie 2, incluant le n° 1355, ont été les dernières à être reliées. Nous n'en faisons désormais plus la reliure ni la distribution. Nous continuons cependant de numéroter les articles et d'en établir des listes d'études sur des recherches mentionnées dans d'autres périodiques. On peut donc se les procurer, sous leur forme réimprimée seulement, de l'auteur ou du service dépositaire.

2039. Sergeant, D. E. 1973. Environment and reproduction in seals. J. Reprod. Fertil. 19: 551–561.

2040. Percy, J. A. 1974. Thermal adaptation in the boreo-arctic echinoid, *Strongylocentrotus droebachiensis* (O. F. Müller, 1776). IV. Acclimation in the laboratory. Physiol. Zool. 47: 163–171. (5)

2041. Freese, M., and M. A. K. Hamid. 1974. Lipid content determination in whole fish using ultrasonic pulse backscatter. Proc. 1974 Ultrasonics Symp., Nov. 11–14, 1974. p. 69–76. (4)

2042. Rosenberg, D. M. 1974. The use of Chironomidae as indicators of macroinvertebrate diversity in a study of pesticide pollution. Proc. 5th Int. Symp. Chironomidae. Ent. Tidskr. 95, Suppl.: 212–215. (4)

2043. Sæther, O. A. 1974. Morphology and terminology of female genitalia in Chironomidae (Diptera). Proc. 5th Int. Symp. Chironomidae. Ent. Tidskr. 95, Suppl.: 216–223. (4)

2044. Conover, R. J., and C. M. Lalli. 1974. Feeding and growth in *Clione limacina* (Phipps), a pteropod mollusc. II. Assimilation, metabolism, and growth efficiency. J. Exp. Mar. Biol. Ecol. 16: 131–154. (7)

2045. Laycock, R. A. 1974. The detrital food chain based on seaweeds. I. Bacteria associated with the surface of *Laminaria* fronds. Mar. Biol. (N.Y.) 25: 223–231. (7)

Translation Series

The following translations have been prepared by or for the Fisheries and Marine Service, for use in its investigations. Copies are available from Translation Services, Canada Institute for Scientific and Technical Information, National Research Council of Canada, Ottawa, Ontario K1A 0S2, at a cost of 10¢ per page and a minimum charge of $1.00. Information on translations can generally be obtained from the Office of the Editor.

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Les traductions dont il est question ci-après ont été préparées par ou pour le Service des pêches et des sciences de la mer, à des fins de recherche. On peut en obtenir des exemplaires, à 10¢ la page et à un prix minimum de $1.00 aux Services de traduction, Institut canadien de l'information scientifique et technologique, Conseil national de recherches du Canada, Ottawa (Ontario), K1A 0S2. Le Bureau de rédacteur peut en général fournir des renseignements sur ces traductions.

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MINDER, L. P. 1954. The curing of salt fish. Tr. Vses. Nauchno-Issled. Inst. Morsk. Rybn. Khoz. Okeanogr. 29: 3–9. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3337, 1975)

NIGMATULLIN, CH. M. 1972. The feeding of ommastrephids off the coast of Africa. Tr. Atl. Nauchno-Issled. Inst. Rybn. Khoz. Okeanogr. (42): 152–155. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3338, 1975)

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Biological Station
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Fisheries and Marine Service
Office of the Editor
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C.P. 429
Halifax (Nouvelle-Ecosse) B3J 2R3

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116, rue Lisgar
Ottawa (Ontario) K1A 0H3

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Service des pêches et des sciences de la mer
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867, ch. Lakeshore
Burlington (Ontario) L7R 4A6

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Service des pêches et des sciences de la mer
Bureau régional de l'Atlantique,
Comité du directeur
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Halifax (Nouvelle-Ecosse) B3J 2M4

ADDITIONAL PUBLICATIONS / PUBLICATIONS ADDITIONNELLES

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The *Annual Report* of the Board and the biennial *Review* of investigations are available free, on request. All publications may be referred to at establishments located at: Ottawa, Ont.; Nanaimo, Vancouver, and West Vancouver, B.C.; Winnipeg, Man.; Ste. Anne de Bellevue, Que.; St. Andrews, N.B.; Halifax and Dartmouth, N.S.; and St. John's, Nfld.

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Journal
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A more detailed guide on preparation of manuscripts from theses is available from the Office of the Editor.

CONTENT

We invite submission of manuscripts in the broad field of fisheries management, ocean science, and aquatic environments relevant to Canada.

Articles: Reports of research studies of broad scope that are original contributions to science.

Notes: Differ from papers on the basis of scope or completeness, rather than quality of research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on methodology or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as articles except that they have no centered headings and should occupy no more than four printed pages.

Reviews: Detailed articles that are critical appraisals of broad areas of investigation, particularly in fields that have shown rapid development in recent years.

Perspectives: Essays of opinion or hypothesis on fisheries, ocean science, aquatic environments, or related topics that are of concern to the professional and layman. References are desirable; informal style is welcomed.

Letters: Opinion on topics recently under discussion in the *Journal*; preferred maximum length, 500 words. The Editor reserves the right to shorten letters and to make other changes to accord with the style of the *Journal.*

EDITORIAL PRACTICES

The *Style Manual for Biological Journals* (3rd ed. American Institute of Biological Sciences, 3900 Wisconsin Ave. NW., Washington, D.C.) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopédique.* Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970) Special Publication No. 6 of the American Fisheries Society.

Series titles should be avoided. An *abstract* is required for all manuscripts, and should state concisely, in less than 250 words, what was done, the essentials of the new knowledge, and the main conclusions. Abstracts are published in English and French; those submitted in one language only are translated by the *Journal.*

The Editor normally submits each manuscript to at least two *referees.* Contributors are encouraged to suggest at least four names to be added to the list from which referees may be selected.

Illustrations should be submitted in triplicate as photographs (*glossy prints*) no larger than a standard page and must accompany the manuscript. Prints should not be mounted, stapled, or clipped. Symbols, letters, and numerals must be neat, and at least 1.5 mm high when the illustration is reduced to the appropriate size. Drawings should, when possible, be grouped to facilitate comparisons.

Proofs are sent to the author, who is responsible for correcting only errors in typesetting. *Alterations to the content must be avoided.*

Fifty *reprints* are supplied free. Additional reprints may be purchased at the time of publication (an order form is sent out with the galley proof). All orders should be sent to the Printing Department, University of Toronto Press, 5201 Dufferin Street, Downsview, Ont. M3H 5T8. Invoices relating to the reprints will be sent out by the Printer. A charge of $30.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

CHECKLIST ON MANUSCRIPTS

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the research?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

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Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

Sujets

Nous désirons reçevoir des manuscrits s'insérant dans le vaste domaine de la gestion des pêches, des sciences de la mer et des milieux aquatiques au Canada.

Articles: Comptes rendus d'études d'une portée générale, constituant des contributions inédites à la science.

Notes: Se distinguent des articles par la portée et le degré d'achèvement plutôt que par la qualité des recherches. Elles peuvent traiter de problèmes particuliers et bien définis; de travaux qui sont en grande partie confirmatoires; de méthodologie ou de techniques de recherche; de progrès de la connaissance découlant d'études plus vastes; de développements dans des projets abandonnés; ou de découvertes préliminaires qui méritent d'être publiées avant la solution de problèmes à l'étude. Les notes peuvent être présentées d'une façon aussi souple que les articles, mais elles ne doivent pas porter d'en-têtes centrés ni occuper plus de quatre pages.

Critiques: Articles détailés constituant une appréciation critique de vastes champs de recherches, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives: Essais de l'opinion ou de l'hypothèse sur les pêches, les sciences de la mer, les milieux aquatiques, ou sur des sujets connexes qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans un style simple.

Lettres: Opinions sur des sujets exposés dans le *Journal*. Longueur maximale préférable: 500 mots. Le directeur se réserve le droit de raccourcir les lettres et de faire d'autres modifications dans le style du *Journal*.

Dispositions générales

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Il faut éviter les *articles en série*. Un *résumé* doit accompagner chaque texte et doit exposer brièvement (moins de 250 mots) le travail accompli, les points essentiels des connaissances acquises et les principales conclusions. Les résumés paraissent en anglais et en français, le *Journal* veillant à la traduction dans le cas des présentations unilingues.

Le directeur soumet d'ordinaire chaque texte à l'approbation de deux *arbitres* ou plus. Nous encourageons les collaborateurs à suggérer au moins quatre personnes dont les noms pourraient figurer sur la liste des arbitres.

Les *illustrations* doivent être sous forme de photos (*sur papier glacé*), ne pas dépasser les dimensions d'une page normale et accompagner le texte. Elles ne doivent pas être montées, coupées ni brochées. Les symboles, lettres ou chiffres doivent se détacher avec précision et avoir au moins 1,5 mm de hauter lorsque l'illustration est réduite à la grandeur voulue. Les dessins doivent, autant que possible, être groupés pour faciliter l'étude comparative.

L'auteur doit revoir les *épreuves* qu'on lui remet et en corriger seulement les erreurs typographiques. *Il doit éviter de changer le contenu du texte.*

Cinquante *tirés à part* sont fournis gratuitement. Les tirés à part peuvent être achetés au moment de la publication. Un bon de commande à cet effet accompagne les épreuves; il doit être retourné en même temps que celles-ci, non pas au bureau de la rédaction, mais au Printing Department, University of Toronto Press, 5201 Dufferin Street, Downsview, Ont. M3H 5T8, qui se chargera de la facturer. Les frais sont de $30.00 par page pour la publication des articles. Quand un auteur soumet un manuscrit, il doit spécifier que son institution accepte les frais.

Listes de contrôles pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphs son-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

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Guide for Typists Preparing Copy

Read "Guide for Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and two other clear copies to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C).

Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered. Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send at least 3 *glossy prints* (no larger than 21.5 × 28 cm) of each illustration with the manuscript. Type the author's name and figure number on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the numeral *one*, and between the capital letter *O* and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

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Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21,5 × 28 cm (8,5 × 11 pouces), laissant toujours une marge d'au moins 2,5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les miniscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, ne nom et l'affiliation des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et ainsi que deux copies lisibles au bureau du Directeur de la publication.

Tableaux. Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).

Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*.

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par les minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Envoyez avec le manuscrit trois photos *sur papier glacé* (21,5 × 28 cm ou moins) pour chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le *Journal* (voir Appendice E). Les noms de revues sont abrégés suivants les *Biological Abstracts*; en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

Recent Bulletins/Récents Bulletins

183 Russian–English dictionary for students of fisheries and aquatic biology
W. E. RICKER
(1973, 428 p., cat. no. Fs 94-183; Canada: $7.00, Other countries/Autres pays: $8.40)

184 Freshwater fishes of Canada
W. B. SCOTT AND E. J. CROSSMAN
(1973, 966 p., cat. no. Fs 94-184; Canada: $9.75, Other countries: $11.70)

184F Poissons d'eau douce du Canada
W. B. SCOTT ET E. J. CROSSMAN
(1974, 1026 p., n° de cat. Fs 94-184F; Canada: $9.75, Autres pays: $11.70)

185 Benthic errantiate polychaetes of British Columbia and Washington
KARL BANSE AND KATHARINE D. HOBSON
(1974, 111 p., cat. no. Fs 94-185; Canada: $4.50, Other countries/Autres pays: $5.40)

186 The capelin (*Mallotus villosus*): biology, distribution, exploitation, utilization, and composition
P. M. JANGAARD
(1974, 70 p., cat. no. Fs 94-186; Canada: $3.00, Other countries/Autres pays: $3.60)

186F Le capelan (*Mallotus villosus*): biologie, distribution, exploitation, utilisation et composition
P. M. JANGAARD
(1975, 77 p., n° de cat. Fs 94-186F; Canada: $3.00; Autres pays $3.60)

187 The lobster fishery of the Maritime Provinces: economic effects of regulations
A. GORDON DEWOLF
(1974, 59 p., cat. no. Fs 94-187; Canada: $3.00, Other countries/Autres pays: $3.60)

188 Aquaculture in Canada — the practice and the promise
H. R. MACCRIMMON, J. E. STEWART, AND J. R. BRETT
(1974, 84 p., cat. no. Fs 94-188; Canada: $3.75, Other countries/Autres pays: $4.50)

189 Treatment of fish processing plant wastewater
F. G. CLAGGETT AND J. WONG
(1974, 18 p., cat. no. Fs 94-189; Canada: $2.00, Other countries/Autres pays: $2.40)

190 Hydrodynamics and energetics of fish propulsion
PAUL W. WEBB
(1975, 159 p., cat. no. Fs 94-190; Canada: $5.00, Other countries/Autres pays: $6.00)

191 Computation and interpretation of biological statistics of fish populations
W. E. RICKER
(1975, 382 p., cat. no. Fs 94-191; Canada: $8.00, Other countries/Autres pays: $9.60)

192 Catalogue and synopsis of *Caligus*, a genus of Copepoda (Crustacea) parasitic on fishes
L. MARGOLIS, Z. KABATA, AND R. R. PARKER
(1975, 117 p., cat. no. Fs 94-192; Canada: $5.00, Other countries/Autres pays: $6.00)

To obtain the publications listed above, at the prices indicated, write to:

Information Canada
171 Slater Street
Ottawa, Canada K1A 0S9

Please make cheques and money orders payable to the Receiver General of Canada. Advance payment is required.

On peut se procurer les publications ci-dessus, au prix indiqué, en s'adressant directement à:

Information Canada
171, rue Slater
Ottawa, Canada K1A 0S9

Payables à l'avance. Faire les chèques et les mandats payables à l'ordre du Receveur général du Canada.

Journal of the Fisheries Research Board of Canada

Journal de l'office des recherches sur les pêcheries du Canada

JFRBAK32(6)729–873(1975)

Volume 32, No. 6, June 1975

Volume 32, n° 6, juin 1975

Chemical attraction of some marine fish in their natural habitat **A. M. Sutterlin** 729–738

Quantitative and qualitative studies of bacteria isolated from sea water used in the laboratory culture of the American oyster, *Crassostrea virginica* **R. A. Murchelano, C. Brown, and J. Bishop** 739–745

Utilization of blue whiting, *Micromesistius poutassou*, for human consumption **B. Dagbjartsson** 747–751

Effects of acute exposure to bleached kraft pulpmill effluent on carbohydrate metabolism of juvenile coho salmon (*Oncorhynchus kisutch*) during rest and exercise **D. J. McLeay and D. A. Brown** 753–760

Density of the commercial spider crab, *Chionoecetes opilio*, and calibration of effective area fished per trap using bottom photography **R. J. Miller** 761–768

Effects of pH on toxicity of antimycin on fish **L. L. Marking** 769–773

Protein instability in minced flesh from fillets and frames of several commercial Atlantic fishes during storage at −5 C **J. R. Dingle and J. A. Hines** 775–783

Effect of DDT on discriminating ability of rainbow trout (*Salmo gairdneri*) **P. G. McNicholl and W. C. Mackay** 785–788

Swimming performance of juvenile coho salmon (*Oncorhynchus kisutch*) exposed to bleached kraft pulpmill effluent **T. E. Howard** 789–793

Mercury in seals, terrestrial carnivores, and principal food items of the Inuit, from Holman, N.W.T. **T. G. Smith and F. A. J. Armstrong** 795–801

Notes

Selenium toxicity on the early life stages of zebrafish (*Brachydanio rerio*) **A. J. Niimi and Q. N. LaHam** 803–806

Tocopherol composition of frozen Atlantic herring (*Clupea harengus harengus*) tissues and oil **F. R. Engelhardt, J. R. Geraci, and B. L. Walker** 807–809

Effects of saline from North Dakota lakes on survival of fathead minnow (*Pimephales promelas*) embryos and sac fry **B. L. Burnham and J. J. Peterka** 809–812

Simplified spectrophotometric curcumin method for the determination of boron in marine shellfish **R. E. Drew** 813–816

Effects of kokanee salmon (*Oncorhynchus nerka*) decomposition on the ecology of a subalpine stream **J. E. Richey, M. A. Perkins, and C. R. Goldman** 817–820

Perspectives

Reproductive guilds of fishes: a proposal and definition **E. K. Balon** 821–864

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Netting materials for fishing gear (G. Klust) **P. J. G. Carrothers** 865–866

Modern inshore fishing gear — rigging and mending (J. Garner) **P. J. G. Carrothers** 866

The poisons around us. Toxic metals in food, air, and water (H. A. Schroeder) **J. B. Sprague** 867

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